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Monash University

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**Patents Act 1990**

**PROVISIONAL SPECIFICATION**

for the invention entitled:

"Novel Therapeutic Molecules and Uses Therefor - II"

The invention is described in the following statement:

- 1A -

## NOVEL THERAPEUTIC MOLECULES AND USES THEREFOR - II

The present invention relates generally to novel molecules capable of, *inter alia*, controlling cellular functional activity such as proliferation, differentiation and/or transcriptional regulation and to genetic sequences encoding same. More particularly, the present invention relates to novel members of the ETS family of proteins, referred to herein as "ELF5", and to genetic sequences encoding same. The molecules of the present invention are useful, for example, in therapy, diagnosis, antibody generation and as a screening tool for agents capable of modulating transcriptional events during cellular functioning such as in tumorigenesis.

### Background of the Invention

The ETS family of transcription factors share a conserved DNA binding domain, termed the 'ETS domain', first identified in the *gag-myb-ets* fusion protein of avian leukemia virus E26 (Nunn *et al.*, 1983; Watson *et al.*, 1988; Karim *et al.*, 1990; Gutman and Wasylyk, 1991; Seth *et al.*, 1992). The ETS domain recognises and binds to purine rich GGA(A/T) core motifs in the promoters and enhancers of various target genes (Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Werner *et al.*, 1995; Kodandapani *et al.*, 1996). The ETS family does not maintain overall similarity outside of the ETS domain, but can be grouped into subfamilies based upon variation within the ETS domain, and also by the arrangement and presence of other domains, such as those involved in transactivation and sites of phosphorylation (Lautenberger *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993). Over 30 ETS gene family members have been identified in species ranging from sea urchin to human.

Many ETS factors have been implicated in the control of cellular proliferation and tumorigenesis (Seth *et al.*, 1992; Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Scott *et al.*, 1994a; Muthusamy *et al.*, 1995). *ETS1*, *ETS2*, *ERG2* and *PU.1* are proto-oncogenes with mitogenic and transforming activity when

overexpressed in fibroblasts (Seth *et al.*, 1989; Seth and Papas, 1990; Hart *et al.*, 1995; Moreau-Gachelin *et al.*, 1996). In addition, chromosomal translocations involving ETS family members are associated with different human cancers. *ERG* and *ERGB/FLI1* are fused to the *EWS* gene in t(21;22) and t(11;22) translocations, respectively, in Ewing's sarcoma and other primitive neuroectodermal tumors (Sorensen *et al.*, 1994; Ida *et al.*, 1995). *FEV* is fused to *EWS* in a subset of Ewing's tumors in t(2;22) (Peter *et al.*, 1997). *TEL* is fused to the platelet-derived growth factor receptor beta (PDGFR $\beta$ ) gene in t(5;12) translocations of chronic myelomonocytic leukemia, and to the acute myeloid leukemia 1 (AML1) transcription factor gene in t(12;21) translocations of acute lymphoblastic leukemia (Golub *et al.*, 1994, 1995). Fusion of *TEL* to the receptor-associated kinase JAK2 results in early pre-B acute lymphoid leukemia in t(9;12), and in a typical chronic myelogenous leukemia in t(9;15;12) (Peeters *et al.*, 1997). Expression of *Spil* and *Flil* can be activated by position specific integration of the Friend murine leukemia virus in murine erythroleukemias (Ben-David *et al.*, 1991). Also, ETS1, ETS2 and ERG regulate the expression of metalloproteinase genes, such as stromelysin and collagenase (Buttice and Kurkinen, 1993; Buttice *et al.*, 1996; Wasylyk *et al.*, 1991), which are important for extracellular matrix degradation concomitant with tumor vascularization (angiogenesis) and metastasis.

ETS factors also have important developmental roles. *Pointed P2* and *yan* play critical roles in *Drosophila* eye development (O'Neill *et al.*, 1994). *ETS2* is involved in skeletal/cartilage development (Sumarsono *et al.*, 1996). *PU.1* null mutation results in haematopoietic abnormalities (McKercher *et al.*, 1996), and *ETS1* is involved in transactivation of genes required for T cell function (Muthusamy *et al.*, 1995; Sun *et al.*, 1995; Thomas *et al.*, 1995; Thomas *et al.*, 1997) and angiogenesis (Wasylyk *et al.*, 1991; Vandenbunder *et al.*, 1994; Wernert *et al.*, 1992).

The ETS factors are almost all expressed in haematopoietic lineages (Bhat *et al.*, 1989; Bhat *et al.*, 1990; Kola *et al.*, 1993), and indeed appear to function predominantly in these cells and their related neoplasms. However, the most common solid tumors in humans are

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carcinomas which arise from the transformation of epithelial cells. Transformed breast epithelial cells, for example, have been shown to express ETS family members GABP $\alpha$ , PEA3, ELF1, ETS1 and ELK1 (Scott *et al.*, 1994b; Delannoy-Courdent *et al.*, 1996), but expression of these ETS family members is not restricted to epithelial cells. One ETS  
5 family member, ELF3/ESX/ESE-1/ERT, has recently emerged with epithelial and epithelial-cancer specific expression (Tymms *et al.*, 1997; Chang *et al.*, 1997; Choi *et al.*, 1998; Oettgen *et al.*, 1997).

In work leading up to the present invention, the inventors have identified and sequenced a  
10 novel member of the ETS family, designated herein "ELF5".

### Summary of the Invention

Bibliographic details of the publications referred to by author in this specification are  
15 collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of the SEQ ID NOs. is provided before the Examples.

Throughout this specification and the claims which follow, unless the context requires  
20 otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

One aspect of the present invention provides an isolated nucleic acid molecule comprising a  
25 nucleotide sequence encoding ELF5 wherein said ELF5 comprises an ETS domain.

Another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in one of SEQ ID NO:2 or 4 or a derivative thereof or  
30 having at least about 45% or greater similarity to one or more of SEQ ID NO:2 or 4 or a derivative thereof.

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Yet another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:7 or having at least about 45% or greater similarity to a sequence comprising the amino acid sequence set forth in SEQ ID  
5 NO:7 or a derivative thereof.

Still yet another aspect of the present invention contemplates a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in one of SEQ ID NO:1 or 3 or a derivative thereof capable of hybridising to one of SEQ ID NO:1 or 3 under low stringency  
10 conditions at 42°C and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of SEQ ID NO:2 or 4 or a sequence having at least about 45% similarity to one or more of SEQ ID NO:2 or 4.

A further aspect of the present invention contemplates a nucleic acid molecule comprising a  
15 nucleotide sequence substantially as set forth in one of SEQ ID NO:5 or 6 or a derivative thereof capable of hybridising to one of SEQ ID NO:5 or 6 under low stringency conditions at 42°C and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in SEQ ID NO:7 or a sequence having at least about 45% similarity to SEQ ID  
NO:7.

20

Yet another further aspect of the present invention provides a nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set forth in one of SEQ ID NO:1 or 3 or is a derivative thereof including a nucleotide sequence having similarity to one of SEQ ID NO:1 or 3 and which encodes an amino acid sequence  
25 corresponding to an amino acid sequence set forth in one of SEQ ID NO:2 or 4 or a sequence having at least about 45% similarity to one or more of SEQ ID NO:2 or 4.

Still yet another further aspect of the present invention provides a nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set  
30 forth in one of SEQ ID NO:5 or 6 or is a derivative thereof including a nucleotide sequence having similarity to one of SEQ ID NO:5 or 6 and which encodes an amino acid sequence

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corresponding to an amino acid sequence as set forth in SEQ ID NO:7 or a sequence having at least about a 45% similarity to SEQ ID NO:7.

Another aspect of the present invention contemplates a method of modulating activity of  
5 ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease ELF5 activity.

Yet another aspect of the present invention contemplates a method of modulating cellular  
10 functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleotide sequence encoding *ELF5* or sufficient to modulate the activity of *ELF5*.

15 Still yet another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of *ELF5* or *ELF5*.

A further aspect of the present invention relates to a method of treating a mammal said  
20 method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of *ELF5* wherein said modulation results in modulation of cellular functional activity.

25 Yet another further aspect of the present invention relates to a method of treating a mammal said method comprising administering to said mammal an effective amount of *ELF5* or *ELF5* for a time and under conditions sufficient to modulate cellular functional activity.

Still yet another further aspect of the present invention relates to the use of an agent capable  
30 of modulating the expression of *ELF5* or modulating the activity of *ELF5* in the manufacture of a medicament for the modulation of cellular functional activity.



Another aspect of the present invention relates to the use of ELF5 or *ELF5* in the manufacture of a medicament for the modulation of cellular functional activity.

Yet another aspect of the present invention relates to agents for use in modulating *ELF5*  
5 expression or ELF5 activity wherein said modulation results in modulation of cellular functional activity.

Still yet another aspect of the present invention relates to ELF5 or *ELF5* for use in modulating cellular functional activity.

10

A further aspect of the present invention contemplates a pharmaceutical composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity together with one or more pharmaceutically acceptable carriers and/or diluents. *ELF5*, ELF5 or said agent are referred to as the active ingredients.

15

Another further aspect of the present invention contemplates a method for detecting ELF5 or *ELF5* mRNA in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for ELF5 or *ELF5* mRNA or its derivatives or homologs for a time and under conditions sufficient for an antibody-ELF5 or antibody-ELF5  
20 mRNA complex to form, and then detecting said complex.

Single and three letter abbreviations used throughout the specification are defined in Table 1.

TABLE 1

5                      **Single and three letter amino acid abbreviations**

Amino Acid	Three-letter Abbreviation	One-letter Symbol
10 Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
15 Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
20 Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
25 Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
30 Any residue	Xaa	X

### Brief Description of the Drawings

**Figure 1** is a schematic representation of murine *ELF5* cDNA sequence and relationship to mRNA transcripts. (a) The nucleotide sequence of murine *ELF5* is shown. Breaks in the sequence indicate the source of sequence data; the central region (92-1528) was sequenced from lambda clones, and 5 prime and 3 prime were added from sequencing of RACE PCR products. Numbering of the nucleotides, starting with the most 5-prime sequences obtained, are indicated on the right. The open reading frame (ORF) is shown in capital letters, with the initiating start and stop codons underlined. A stop codon, in the same reading frame as the ORF, but 5 prime to the initiating codon, is also underlined. The ETS domain is indicated in a shaded box. Putative polyadenylation signals are underlined with dashed lines. A/T rich tracts in the 3 prime untranslated region are boxed. (b) Northern blot analysis of day 14 mouse placenta: lane 1, probed with random-prime-labeled 940 bp *Sty1* murine *ELF5* cDNA fragment (probe 1); lane 2, probed with random-prime-labeled murine *ELF5* 696 bp 3'-RACE PCR product (probe 2). Positions of 28S and 18S markers are indicated. Both lanes were also probed with GAPDH cDNA (lower panels).

**Figure 2** is a schematic representation of (a) Comparison of human and mouse ORFs. Amino acid sequences present in both human and mouse *ELF5* are shaded. The ETS domain is boxed with a solid line and the pointed domain with a dashed line. Putative phosphorylation sites, conserved between the two species are circled and labeled as CKII (casein kinase II), PKC (protein kinase C) or TyP (tyrosine kinase) substrates. (b) Comparison of the ETS domain of human and mouse *ELF5* with those of known members of the ETS gene family. The alignment was generated using CLUSTAL W (Thompson *et al.*, 1994) with the default settings, and the result was subsequently adjusted manually. The ETS factors examined are labeled on the left and include hELF3, mELF3, hNERF, dETS4, dE74A, hELF1, hELK1, hTEL, hERM, mER81, mPEA3, mGABP, mERP, dETS6, mPU1, hPE1, hSAP1, hSPIB, dYAN, hERG, mFLI1, dELG, dETS3, mETS1, mETS2, mER71, where 'h' denotes human, 'm' mouse and 'd' *Drosophila*. The ETS consensus sequence is a list of the amino acids most often conserved between ETS family members. Shading denotes amino acid identity with human *ELF5*, and the percent identity of each ETS domain is

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- indicated on the right. (c) Phylogenetic tree of the ETS domain produced by maximum likelihood analysis. The alignment in Figure 2b was analysed using the JTT-F substitution model (Jones *et al.*, 1992) and local bootstrap values were estimated for all internal branches, both by using PROTML in Q mode followed by a second run in R mode (Adachi and
- 5 Hasegawa, 1996). An underlying assumption of the phylogenetic analysis is that the amino acid content does not vary significantly among the sequences. This assumption was not assessed because tools for doing so are still under development (LSJ, unpublished work). Therefore, the tree may be the result of both historical and compositional components. The four points at which gene duplications have been inferred are marked A, B, C and D. (d)
- 10 Comparison of the pointed domain of human and mouse ELF5 with those of other members of the ETS family. The ETS factors examined are labeled on the left and include hERG, hELF3, hTEL, hGABP $\alpha$ , hETS1, hETS2, dYAN and dPOINTEDP2. Other labels and conventions are as described for Figure 2b.
- 15 **Figure 3** is a schematic representation of the chromosomal localization of human *ELF5*. Human chromosomal localization of ELF5 was performed by PCR using gene specific primers and the Genebridge 4 Radiation Hybrid DNA panel (UK HGMP Resource Centre). Diagram based upon PCR results (data not shown) showing localization of *ELF5* within chromosome 11, with respect to adjacent marker obtained from mapping data (see text).
- 20 **Figure 4** is a photographic representation of *ELF5* expression in mouse tissues. Positions of 28S and 18S markers are indicated. *ELF5a* and *ELF5b* transcripts are indicated. (a) Northern analysis of adult mouse tissues probed with murine *ELF5* cDNA (top panels) and GAPDH cDNA (lower panels). Abbreviations; Li: liver; Lu: lung; Br: brain; Ki: kidney; He: heart; Sm: small intestine; Sp: spleen; Th: thymus; St: stomach; Ov: ovary; Pa: pancreas; To: tongue; Sk: skeletal muscle; Bl: bladder; 2Fa: day 2 pregnant fat; 2 Ma: day 2 pregnant mammary gland; 10 Fa: day 10 pregnant fat; 10 Ma: day 10 pregnant mammary gland; Co: colon. Arrow indicates position of brain specific transcript (see text). (b) Northern analysis as above, but using RNA from day 1 neonate mouse tissues. Additional abbreviation; In:
- 30 intestine. Arrow indicates position of large transcript (see text). (c) Northern analysis as above, but using RNA from day 16, 17 and 19 embryonic tissues. (d) Northern analysis as

above, but using RNA from day 9.5 to day 19 placental tissues as indicated.

**Figure 5** is a photographic representation of *ELF5* expression in human tissues and cell lines. (a) Northern analysis of adult human tissues probed with human *ELF5* cDNA (top panels) and  $\beta$ -Actin cDNA (lower panels). The single *ELF5* transcript is indicated. Other labels and conventions are as for Figure 4. Abbreviations; He: heart; Br: brain; Pl: placenta; Lu: lung; Li: liver; Sk: skeletal muscle; Ki: kidney; Pa: pancreas; Sp: spleen; Th: thymus; Pr: prostate; Te: testis; Ov: ovary; Sm: small intestine; Co: colon mucosa; PBL: peripheral blood lymphocytes. (b) RNase protection analysis of *ELF5* and GAPDH in cell lines; 1: CaOv-3 (ovarian carcinoma); 2: BT-549 (ductal breast carcinoma); 3: ZR-75-1 (breast carcinoma); 4: T47D (ductal breast carcinoma, progesterone sensitive); 5: 786-O (renal adenocarcinoma); 6: SK-HEP-1 (liver adenocarcinoma); 7: A549 (lung adenocarcinoma); 8: CCL32SK (primary fibroblast); 9: MEL28 (melanoma); 10: WISH (amnion carcinoma); 11: Jurkat (T cell leukemia); 12: DU145 (prostate carcinoma); 13: PC3 (prostate carcinoma); 14: HEC-1 (endometrium carcinoma); 15: K562 (erythroid leukemia). (c) Southern analysis of *ELF5* in *Bgl*III digested genomic DNA from cell lines; 1: normal blood; 2: BT-549 (ductal breast carcinoma); 3: ZR-75-1 (breast carcinoma); 4: T47D (ductal breast carcinoma); 5: NCI-H1299 (large cell lung carcinoma); 6: NCI-H187 (small cell lung carcinoma); 7: NCI-H322 (bronchioalveolar carcinoma); 8: NCI-H358 (bronchioalveolar carcinoma); 9: NCI-H522 (lung adenocarcinoma); 10: SK-LU-1 (lung adenocarcinoma); 11: NCI-H441 (bronchioalveolar carcinoma); 12: NCI-H460 (large cell lung carcinoma); 13: NCI-H661 (large cell lung carcinoma).

**Figure 6** is a photographic representation of *ELF5* binding to consensus ETS binding sequences. (a) His-tagged *ELF5* recombinant protein, present in *E. coli* lysates (lane 2), was purified by metal-affinity chromatography to approximately 90% (lane 3) and eluted with imadazole (lane 4). (b) Specific DNA binding of *Elf5* was analysed by electrophoretic mobility shift assay (EMSA), using labeled double-stranded oligonucleotides as probes. E74 contains a consensus binding site for ETS family members (lane 1). E74ml is a mutant oligonucleotide based on E74, but with the core GGAA replaced by AGAA (lane 2). Binding to other consensus ETS sites was analysed by the ability of a 100-fold excess of unlabeled

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double-stranded oligonucleotide to compete with E74 for Elf5 binding GMETS contains an ETS binding site from the human GM-CSF promoter (lane 6). ERBB2 contains an ETS binding site from the human *erbB2*/HER2 promoter (lane 7). MSV contains an ETS binding site present in the long terminal repeat of the Moloney sarcoma virus (lane 8). AP1 contains a consensus AP1 binding site used as a negative control ELF5-DNA complexes are marked. Binding of ETS1 to E74 was used as a positive control (lane 10).

**Figure 7** is a graphical representation of transactivation by ELF5. COS cells were co-transfected with CAT reporter and Elf5 expression constructs. Transcription of the CAT gene was driven by the thymidine kinase (tk) minimal promoter with five copies of the polyomavirus enhancer inserted upstream (p5Xpoly). The polyomavirus enhancer contains adjacent ETS and AP1 binding sites. The ELF5 sense construct (pBOSElf5as) was designed to express ELF5 protein, and the *ELF5* anti-s construct (pBOSElf5as) to produce anti-sense transcripts. In the absence of expression construct the equivalent amount of base vector (pEFBOS) was co-transfected. COS cells were processed for CAT assays and the results of at least four replicates are shown as the mean with standard error of the mean (s.e.m.) bars. Statistically significant results are indicated by asterisks. A single asterisk indicates moderate significance ( $0.05 > P > 0.01$ ) and triple asterisks indicate very high significance ( $P < 0.001$ ).

**Figure 8** is a photographic representation of breast tissue sections from paraffin-embedded samples which had been hybridized with ELF5 antisense RNA.

### Detailed Description of the Invention

The present invention is predicated, in part, on the identification of a novel member of the ETS family of molecules, termed ELF5. The identification of this novel molecule permits the  
5 identification and rational design of a range of products for use in therapy, diagnosis and antibody generation involving, for example, regulation of cellular functional activity such as cellular proliferation. These therapeutic molecules may also act as either antagonists or agonists of ELF5 function and will be useful, *inter alia*, in cancer and autoimmune disease therapy.

10

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding ELF5 wherein said ELF5 comprises an ETS domain.

15 Reference to an "ETS domain" should be understood as a reference to a protein domain which recognises and binds to a purine rich GGA(A/T) motif of a promoter or enhancer (Macleod *et al.*, 1992; Wasylyk *et al.*, 1993; Janknecht and Nordheim, 1993; Werner *et al.*, 1995; Kodandapani *et al.*, 1996). The ETS domain may be continuous, meaning that it is comprised of a continuous sequence of amino acids, or it may be discontinuous, meaning that  
20 it is comprised of individual amino acids or sequences of amino acids from two or more separate regions of the protein and which are brought into proximity with one another to form the ETS domain due to the secondary, tertiary or quaternary structure of the protein.

More particularly, the present invention provides a nucleic acid molecule comprising a  
25 nucleotide sequence encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in one of SEQ ID NO:2 or 4 or a derivative thereof or having at least about 45% or greater similarity to one or more of SEQ ID NO:2 or 4 or a derivative thereof.

30 Another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding an amino acid

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sequence substantially as set forth in SEQ ID NO:7 or having at least about 45% or greater similarity to a sequence comprising the amino acid sequence set forth in SEQ ID NO:7 or a derivative thereof.

5 The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid levels. Where there is non-identity of the nucleotide level "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particular preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch. Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website <http://mel1.angis.org.au..>

20

Another aspect of the present invention contemplates a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in one of SEQ ID NO:1 or 3 or a derivative thereof capable of hybridising to one of SEQ ID NO:1 or 3 under low stringency conditions at 42°C and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of SEQ ID NO:2 or 4 or a sequence having at least about 45% similarity to one or more of SEQ ID NO:2 or 4.

25

More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:1 or 3.



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Another aspect of the present invention contemplates a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in one of SEQ ID NO:5 or 6 or a derivative thereof capable of hybridising to one of SEQ ID NO:5 or 6 under low stringency conditions at 42°C and which encodes an amino acid sequence corresponding to an amino acid sequence  
 5 set forth in SEQ ID NO:7 or a sequence having at least about 45% similarity to SEQ ID NO:7.

More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or 6.

10

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium  
 15 stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for  
 20 hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions. In general, washing is carried out at  $T_m = 69.3 + 0.41 (G + C) \% [19] = -12^\circ\text{C}$ . However, the  $T_m$  of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatched based pairs (20).

25 The nucleic acid molecule according to this aspect of the present invention corresponds herein to "*ELF5*". This gene has been determined in accordance with the present invention to encode a protein which displays specific binding to DNA sequences comprising a GGA(A/T) core. The product of the *ELF5* gene is referred to herein as ELF5. ELF5 is defined as belonging to the ETS family of transcription factors due to its expression of an ETS domain  
 30 which recognises and binds the purine rich GGA(A/T) core motifs. ELF5 is a protein for which splice variants exist, thereby resulting in the expression of a variety of isoforms.

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Human ELF5 and human ELF5 short transcript are examples of 2 isoforms which differ in size due to the splicing out of exon regions from the *ELF5* mRNA molecule encoding the ELF5 short transcript. Murine *ELF5a* and *ELF5b* are examples of 2 mRNA transcripts which differ in the length of the 3' untranslated region. Human ELF5 and ELF5 short  
5 transcript are defined by the amino acid sequences set forth in SEQ ID NO: 2 and 4, respectively and murine ELF5 is defined by the amino acid sequence set forth in SEQ ID NO:7. The cDNA nucleotide sequences for human *ELF5* and *ELF5* short transcript are defined by the nucleotide sequences set forth in SEQ ID NO:1 and 3, respectively, and murine *ELF5a* and *ELF5b* are defined by the nucleotide sequences set forth in SEQ ID NO:5  
10 and 6, respectively.

The nucleic acid molecules encoding ELF5 are preferably a sequence of deoxyribonucleic acids such as cDNA sequences or genomic sequences. A genomic sequence may also comprise exons and introns. A genomic sequence may also include a promoter region or  
15 other regulatory region.

Reference hereinafter to "ELF5" and "*ELF5*" should be understood as a reference to all forms of ELF5 and *ELF5*, respectively, including by way of example the two mRNA transcripts, *ELF5a* and *ELF5b*, observed in the mouse. Without limiting the invention in any way,  
20 sequence analysis of murine ELF5 has revealed two discrete polyadenylation signals present in the 3' untranslated region (UTR). The first of these (at 1391 bp) appears to be an overlapping poly(A)<sup>+</sup> recognition signal AATTAA and ATTAATA. The second is a consensus polyadenylation signal, AATAAA, at 2181 bp. Sequence analysis of human *ELF5* has also revealed two mRNA transcripts arising from the splicing out of part of the exon  
25 region. Accordingly, the present invention should be understood to extend to all cDNA and peptide isoforms arising from alternative splicing of ELF5 mRNA. By way of yet another example, reference herein to "ELF5" and "*ELF5*" should also be understood to include reference to its derivatives thereof.

30 The protein and/or gene is preferably from a human, primate, livestock animal (eg. sheep, pig, cow, horse, donkey) laboratory test animal (eg. mouse, rat, rabbit, guinea pig) companion

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animal (eg. dog, cat), captive wild animal (eg. fox, kangaroo, deer), aves (eg. chicken, geese, duck, emu, ostrich), reptile or fish.

The term "protein" should be understood to encompass peptides, polypeptides and proteins.

- 5 The protein may be glycosylated or unglycosylated, phosphorylated or dephosphorylated to various degrees and/or may contain a range of other molecules fused, linked, bound or otherwise associated to the protein such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins. Reference hereinafer to a "protein" includes a protein comprising a sequence of amino acids as well as a protein associated with other molecules
- 10 such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

Derivatives include fragments, parts, portions, chemical equivalents, mutants, homologs, mimetics from natural, synthetic or recombinant sources including fusion proteins.

Derivatives may be derived from insertion, deletion or substitution of amino acids.

- 15 Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of
- 20 one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences including fusions with other peptides, polypeptides or proteins.

- 25 The derivatives of ELF5 include fragments having particular epitopes or parts of the entire ELF5 protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules. For example, ELF5 or derivative thereof may be fused to a molecule to facilitate its entry into a cell. Analogs of ELF5 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their
- 30 derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or

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their analogs. Derivatives of nucleic acid sequences may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable  
5 for use in cosuppression and fusion of nucleic acid molecules.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate;  
10 acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with  $\text{NaBH}_4$ .

15 The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea  
20 formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride  
25 or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

- 10 Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated  
15 herein is shown in Table 2.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
20 $\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
25 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
30 D-alanine	Dal	L-N-methylleucine	Nmleu

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	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
5	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
10	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
15	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgab
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
20	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
	D- $\alpha$ -methylassparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylasspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
25	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methyllleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
30	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn

	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
5	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- $\alpha$ -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
10	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
15	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
20	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dmthr
25	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- $\alpha$ -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
30	L-ethylglycine	Etg	penicillamine	Pen

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	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylasparagine	Masn
	L- $\alpha$ -methylaspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
5	L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- $\alpha$ -methyllleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
	L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
10	L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
	L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
	L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
	L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
	L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph
15	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
	carbanylmethyl)glycine		carbanylmethyl)glycine	
	1-carboxy-1-(2,2-diphenyl-	Nmbc		
	ethylamino)cyclopropane			

- 
- 20 Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having (CH<sub>2</sub>)<sub>n</sub> spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or
- 25 dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C $\alpha$  and N $\alpha$ -methylamino acids, introduction of double bonds between C $\alpha$  and C $\beta$  atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side
- 30 chain and the N or C terminus.



The nucleic acid molecule of the present invention is preferably in isolated form or ligated to a vector, such as an expression vector. By "isolated" is meant a nucleic acid molecule having undergone at least one purification step and this is conveniently defined, for example, by a composition comprising at least about 10% subject nucleic acid molecule, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 40-50%, even still more preferably at least about 60-70%, yet even still more preferably 80-90% or greater of subject nucleic acid molecule relative to other components as determined by molecular weight, encoding activity, nucleotide sequence, base composition or other convenient means. The nucleic acid molecule of the present invention may also be considered, in a preferred embodiment, to be biologically pure.

In a particularly preferred embodiment, the nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set forth in one of SEQ ID NO:1 or 3 or is a derivative thereof including a nucleotide sequence having similarity to one of SEQ ID NO:1 or 3 and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in one of SEQ ID NO:2 or 4 or a sequence having at least about 45% similarity to one or more of SEQ ID NO:2 or 4.

In another particularly preferred embodiment, the nucleotide sequence corresponding to *ELF5* is a cDNA sequence comprising a sequence of nucleotides as set forth in one of SEQ ID NO:5 or 6 or is a derivative thereof including a nucleotide sequence having similarity to one of SEQ ID NO:5 or 6 and which encodes an amino acid sequence corresponding to an amino acid sequence as set forth in SEQ ID NO:7 or a sequence having at least about a 45% similarity to SEQ ID NO:7.

A derivative of the nucleic acid molecule of the present invention also includes nucleic acid molecules capable of hybridising to the nucleotide sequences as set forth in one of SEQ ID NO:1 or 3 or SEQ ID NO:5 or 6 under low stringency conditions. Preferably said low stringency is at 42°C.

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The nucleic acid molecule may be ligated to an expression vector capable of expression in a prokaryotic cell (eg. *E. coli*) or a eukaryotic cell (eg. yeast cells, fungal cells, insect cells, mammalian cells or plant cells). The nucleic acid molecule may be ligated or fused or otherwise associated with a nucleic acid molecule encoding another entity such as, for  
5 example, a signal peptide, a cytokine or other member of the ETS family.

The expression product is ELF5 having an amino acid sequence set forth in one of SEQ ID NO:2 or 4 or SEQ ID NO:7 or is a derivative or homologue as hereinbefore defined or is a mammalian homologue having an amino acid sequence of at least about 45% similarity to  
10 the amino acid sequence set forth in one of SEQ ID NO:2 or 4 or SEQ ID NO:7 or derivative or homologue thereof.

The ELF5 of the present invention may be in multimeric form meaning that two or more molecules are associated together. Where the same ELF5 molecules are associated  
15 together, the complex is a homomultimer. An example of a homomultimer is a homodimer. Where at least one ELF5 is associated with at least one non-ELF5 molecule, then the complex is a heteromultimer such as a heterodimer. A heteromultimer may include a molecule of another member of the ETS family or other molecule capable of modulating transcription.

20

In accordance with the present invention, it is proposed that ELF5 is a molecule which regulates cellular functional activity. Reference to cellular "functional activity" should be understood as a reference to the functions which a cell is capable of performing such as, but in no way limited to, one or more of proliferation, differentiation, cell surface molecule  
25 expression, antigen presentation, maintenance of viability, apoptosis, metabolism, signal transduction and molecular mechanisms such as transcription and translation. Without limiting this invention to any one theory or mode of action, human *ELF5* has been mapped to human chromosome 11p13-15 which is a region that frequently undergoes loss of heterozygosity in several types of carcinoma, including breast, kidney and prostate  
30 carcinomas. The expression pattern of *ELF5* and ELF5 in normal and diseased tissues also supports a role for these molecules in the regulation of cellular functional activity and, in

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particular, in the direct or indirect regulation of tumorigenesis. Even more particularly, it is proposed that ELF5 functions as a transcription factor.

- The cloning and sequencing of this gene and its expression product now provides an
- 5 additional gene for use in the prophylactic and therapeutic treatment of diseases such as those involving aberrant cellular functional activity such as aberrant cellular proliferation. Examples of diseases involving aberrant cellular proliferation include diseases caused by excessive cellular proliferation, such as in tumorigenesis, or diseases caused by inadequate cellular proliferation. Accordingly, the present invention contemplates therapeutic and
- 10 prophylactic uses of ELF5 amino acid and nucleic acid molecules, in addition to ELF5 agonistic and antagonistic agents, for the regulation of cellular functional activity, such as for example, regulation of proliferation, differentiation and/or regulation of gene expression by transcriptional regulation.
- 15 The present invention contemplates, therefore, a method for modulating expression of *ELF5* in a subject, said method comprising contacting *ELF5* gene with an effective amount of an agent for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *ELF5*. For example, *ELF5* antisense sequences such as oligonucleotides may be introduced into a cell to down-regulate one or more specific
- 20 functional activities of that cell. Conversely, a nucleic acid molecule encoding ELF5 or a derivative thereof may be introduced to up-regulate one or more specific functional activities of any cell not expressing the endogenous *ELF5* gene.

- Another aspect of the present invention contemplates a method of modulating activity of
- 25 ELF5 in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease ELF5 activity.

- Modulation of said activity by the administration of an agent to a mammal can be achieved
- 30 by one of several techniques, including but in no way limited to introducing into said mammal a proteinaceous or non-proteinaceous molecule which:

- 25 -

- (i) modulates expression of *ELF5*;
- (ii) functions as an antagonist of *ELF5*;
- 5 (iii) functions as an agonist of *ELF5*.

Said proteinaceous molecule may be derived from natural or recombinant sources including fusion proteins or following, for example, natural product screening. Said non-proteinaceous molecule may be, for example, a nucleic acid molecule or may be derived  
10 from natural sources, such as for example natural product screening or may be chemically synthesised. The present invention contemplates chemical analogs of *ELF5* capable of acting as agonists or antagonists of *ELF5*. Chemical agonists may not necessarily be derived from *ELF5* but may share certain conformational similarities. Alternatively, chemical agonists may be specifically designed to mimic certain physiochemical properties of *ELF5*.  
15 Antagonists may be any compound capable of blocking, inhibiting or otherwise preventing *ELF5* from carrying out its normal biological functions. Antagonists include monoclonal antibodies specific for *ELF5*, or parts of *ELF5*, and antisense nucleic acids which prevent transcription or translation of *ELF5* genes or mRNA in mammalian cells.

20 Said proteinaceous or non-proteinaceous molecule may act either directly or indirectly to modulate the expression of *ELF5* or the activity of *ELF5*. Said molecule acts directly if it associates with *ELF5* or *ELF5* to modulate the expression or activity of *ELF5* or *ELF5*. Said molecule acts indirectly if it associates with a molecule other than *ELF5* or *ELF5* which other molecule either directly or indirectly modulates the expression or activity of *ELF5* or  
25 *ELF5*. Accordingly, the method of the present invention encompasses the regulation of *ELF5* or *ELF5* expression or activity via the induction of a cascade of regulatory steps which lead to the regulation of *ELF5* or *ELF5* expression or activity.

Another aspect of the present invention contemplates a method of modulating cellular  
30 functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the

expression of a nucleotide sequence encoding *ELF5* or sufficient to modulate the activity of *ELF5*.

Yet another aspect of the present invention contemplates a method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of *ELF5* or *ELF5*.

The *ELF5*, *ELF5* or agent used may also be linked to a targeting means such as a monoclonal antibody, which provides specific delivery of the *ELF5*, *ELF5* or agent to the target cells.

In a preferred embodiment of the present invention, the *ELF5*, *ELF5* or agent used in the method is linked to an antibody specific for said target cells to enable specific delivery to these cells.

Administration of the *ELF5*, *ELF5* or agent, in the form of a pharmaceutical composition, may be performed by any convenient means. *ELF5*, *ELF5* or agent of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the *ELF5*, *ELF5* or agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of *ELF5* or agent may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The *ELF5* or agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules). With particular reference to use of *ELF5* or agent, these peptides may be administered in the form of pharmaceutically acceptable

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nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active  
5 ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

A further aspect of the present invention relates to the use of the invention in relation to  
10 mammalian disease conditions. For example, the present invention is particularly useful, but in no way limited to, use in cancer therapy.

Accordingly, another aspect of the present invention relates to a method of treating a mammal said method comprising administering to said mammal an effective amount of an  
15 agent for a time and under conditions sufficient to modulate the expression of *ELF5* or sufficient to modulate the activity of *ELF5* wherein said modulation results in modulation of cellular functional activity.

In another aspect the present invention relates to a method of treating a mammal said  
20 method comprising administering to said mammal an effective amount of *ELF5* or *ELF5* for a time and under conditions sufficient to modulate cellular functional activity.

Yet another aspect of the present invention relates to the use of an agent capable of modulating the expression of *ELF5* or modulating the activity of *ELF5* in the manufacture  
25 of a medicament for the modulation of cellular functional activity.

A further aspect of the present invention relates to the use of *ELF5* or *ELF5* in the manufacture of a medicament for the modulation of cellular functional activity.

30 Still yet another aspect of the present invention relates to agents for use in modulating *ELF5* expression or *ELF5* activity wherein said modulation results in modulation of cellular

functional activity.

Another aspect of the present invention relates to ELF5 or *ELF5* for use in modulating cellular functional activity.

5

In a related aspect of the present invention, the mammal undergoing treatment may be human or an animal in need of therapeutic or prophylactic treatment.

In yet another further aspect the present invention contemplates a pharmaceutical  
10 composition comprising *ELF5*, ELF5 or an agent capable of modulating *ELF5* expression or ELF5 activity together with one or more pharmaceutically acceptable carriers and/or diluents. *ELF5*, ELF5 or said agent are referred to as the active ingredients.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions  
15 (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion  
20 medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of  
25 microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example,  
30 aluminum monostearate and gelatin.

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Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle  
5 which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

10

When *ELF5*, ELF5 and ELF5 modulators are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic  
15 administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the  
20 weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1  $\mu\text{g}$  and 2000 mg of active compound.

25 The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil  
30 of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may



contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, 5 methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

10

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is 15 incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for 20 ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by 25 and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

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- The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5  $\mu$ g to about 2000 mg. Expressed
- 5 in proportions, the active compound is generally present in from about 0.5  $\mu$ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.
- 10 The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *ELF5* expression or ELF5 activity. The vector may, for example, be a viral vector.
- 15 Still another aspect of the present invention is directed to antibodies to ELF5 including catalytic antibodies. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to ELF5 or may be specifically raised to ELF5. In the case of the latter, ELF5 may first need to be associated with a carrier molecule. The antibodies and/or recombinant ELF5 of the present invention are
- 20 particularly useful as therapeutic or diagnostic agents. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for
- 25 immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regime.

For example, ELF5 can be used to screen for naturally occurring antibodies to ELF5. These may occur, for example in some degenerative disorders.

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For example, specific antibodies can be used to screen for ELF5 proteins. The latter would be important, for example, as a means for screening for levels of ELF5 in a cell extract or other biological fluid or purifying ELF5 made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and  
5 include, for example, sandwich assays, ELISA and flow cytometry.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first  
10 antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of ELF5.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the protein or peptide derivatives and either type is utilizable for immunoassays. The methods of  
15 obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of ELF5, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are  
20 generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an  
25 immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol II, ed. by Schwartz, 1981; Kohler and Milstein, *Nature* 256: 495-499, 1975; *European Journal of Immunology* 6: 511-519, 1976).

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In another aspect of the present invention, the molecules of the present invention are also useful as screening targets for use in applications such as the diagnosis of disorders which are regulated by ELF5. For example, screening for the levels of ELF5 protein or *ELF5* mRNA transcripts in breast or prostate tissue as an indicator of a predisposition to, or the  
5 development of, breast or prostate cancer.

Yet another aspect of the present invention contemplates a method for detecting ELF5 or *ELF5* mRNA in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for ELF5 or *ELF5* mRNA or its derivatives or  
10 homologs for a time and under conditions sufficient for an antibody-ELF5 or antibody-ELF5 mRNA complex to form, and then detecting said complex.

The presence of ELF5 may be determined in a number of ways such as by Western blotting, ELISA or flow cytometry procedures. ELF5 mRNA may be detected, for example, by *in*  
15 *situ* hybridization or Northern blotting. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

20 Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of  
25 incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a  
30 signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample

- containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention
- 5 the sample is one which might contain Bim including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.
- 10 In the typical forward sandwich assay, a first antibody having specificity for the ELF5 or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for
- 15 conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit
- 20 present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.
- 25 An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed
- 30 to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

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By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or  
5 radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the  
10 skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates,  
15 which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which  
20 may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

25 Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As  
30 in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then

exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent

5 molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect *ELF5* or its derivatives.

10 Further features of the present invention are more fully described in the following examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention. It should not be understood in any way as a restriction on the broad description of the invention as set out above.

15

#### SUMMARY OF SEQ ID Nos.

	<u>Sequence</u>	<u>SEQ ID NO.</u>
20	nucleotide sequence of human ELF5	1
	amino acid sequence of human ELF5	2
	nucleotide sequence of human ELF5 short transcript	3
	amino acid sequence of human ELF5 short transcript	4
25	nucleotide sequence of murine ELF5a	5
	nucleotide sequence of murine ELF5b	6
	amino acid sequence of murine ELF5	7
	oligonucleotide primer	8
	oligonucleotide primer	9
30	oligonucleotide primer	10
	oligonucleotide primer	11

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oligonucleotide primer	12
oligonucleotide primer	13
oligonucleotide primer	14
oligonucleotide primer	15

5



**EXAMPLE 1****ISOLATION OF MOUSE AND HUMAN ELF5 cDNAs**

The murine ELF5 cDNA was isolated from an adult mouse lung cDNA library.

5 Amalgamation of sequence data revealed a 1437 bp sequence with a maximum open reading frame (ORF) of 759 bp, predicted to encode a 253 amino acid protein of approximately 31 kD (Figure 1a). An upstream, in-frame stop codon suggests that this ORF represents the full-length coding sequence of ELF5. Additional 91 bp of 5', and 696 bp of 3' sequences were obtained by reverse transcriptase polymerase chain reaction  
10 (PCR) and rapid amplification of cDNA ends (RACE), using day 14 mouse placental RNA. Sequence analysis revealed two discrete polyadenylation signals present in the 3' untranslated region (UTR). The first of these (at 1391 bp) appears to be an overlapping poly(A)<sup>+</sup> recognition signal, AATTAA and ATTAAAA. The second is a consensus polyadenylation signal, AATAAA, at 2181 bp. These polyadenylation signals are found  
15 close to the 3' termination of the original clone and the 3' RACE product, respectively, suggesting that these represent polyA signals for two separate mRNA products. Thus, the two predicted ELF5 cDNAs are 2224 bp and 1528 bp long. Northern blot analysis, using the ELF5 coding sequence as a probe, confirmed the presence of two predominant ELF5 transcripts in placental tissue, ELF5a and ELF5b, of approximately 2.5 kb and 1.5  
20 kb respectively. Only ELF5a was identified using a 3' UTR fragment from between the polyadenylation signals as a probe (Figure 1b), indicating that the transcripts differ in 3' UTR sequences.

A human ELF5 cDNA fragment was isolated from a human lung cDNA library  
25 following screening with a cDNA probe containing the coding sequence of mouse ELF5. The full coding sequence of human ELF5 was then obtained by reverse transcriptase PCR and RACE using human placental RNA. Analysis revealed that the ELF5 sequence is predicted to encode a 255 residue amino acid protein.

## EXAMPLE 2

### COMPARISON OF HUMAN AND MOUSE ELF5 AMINO ACID SEQUENCES

The predicted amino acid sequences of human and mouse ELF5 are highly conserved, with approximately 95% identity (Figure 2a). Only a single amino acid substitution was observed within the putative ETS domain of human and mouse ELF5, and most of the other differing amino acid residues in the full-length sequences are conservative substitutions (8/13), suggesting that the two proteins are homologs (i.e. having an inferred common ancestry). Interestingly, human ELF5 does, however, contain an additional two amino acid insertion compared to mouse ELF5. In addition to the ETS domain, other features appear to be conserved between these two sequences. These include a putative 'pointed' domain (Seth *et al.*, 1992; Lautenberger *et al.*, 1992) and several consensus casein kinase II (CKII) (Pinna, 1990), protein kinase C (PKC) (Kishimoto *et al.*, 1985; Woodget *et al.*, 1986) and tyrosine kinase (Patschinsky *et al.*, 1982; Hunter, 1982; Cooper *et al.*, 1984) phosphorylation sites.

The ETS domain found within all members of the ETS family is responsible for sequence-specific DNA binding (Seth *et al.*, 1992; Lautenberger *et al.*, 1992; Wasylyk *et al.*, 1993). The putative ETS domain of human/mouse ELF5, situated at the carboxyl terminal of the protein, is similar to that of human/mouse ELF3, with amino acid identity being 67%. However, this domain is only moderately similar to that of other ETS family members, with the highest amino acid identity being 49% to human NERF, 48% to *Drosophila* ETS4 and E74A, and 46% to human ELF1 and ELK1 (Figure 2b). Sequence identity to other family members is in the range of 44-36%. However, amino acids highly conserved amongst ETS family members (Janknecht and Nordheim, 1993) are well conserved in ELF5 (23/38). Some of these highly conserved residues, such as the three tryptophan residues in the carboxyl half of the ETS domain, have been demonstrated to be structurally critical for DNA binding of other ETS family members (Wang *et al.*, 1992; Wasylyk *et al.*, 1992).

- 40 -

Based on ETS domain similarities, a recent phylogenetic analysis (Graves and Petersen, 1998) has proposed the grouping of ETS factors into subfamilies, one of which is the ELF (E-74-like-factor) subfamily. The ELF subfamily includes *Drosophila* E74A, human ELF1 and NERF. A phylogenetic tree was generated including ELF5 and recently isolated ELF3, by maximum likelihood analysis of the ETS domain (Figure 2c). It shows that the human and mouse ELF5 sequences group most closely with the human and mouse ELF3 sequences, and that both ELF3 and ELF5 are most closely related to *Drosophila* ETS4, E74A and human ELF1 and NERF within the ETS family. Thus, *Drosophila* ETS4, and human/mouse ELF3 and ELF5 may also fall into the ELF subfamily of ETS factors.

The phylogeny in Figure 2c shows the unrooted relationship among 28 ETS domains.

### EXAMPLE 3

#### HUMAN CHROMOSOMAL MAPPING OF ELF5

Human chromosomal localization of ELF5 was performed by PCR, using gene specific primers and the Genebridge 4 Radiation Hybrid DNA panel (UK HGMP Resource Centre). With these primers, a single product of the expected size (234 bp) was amplified from total human DNA. The PCR reactions were then performed separately for each of the individual hybrids. The amplification results from the 93 hybrids were submitted to the Radiation Hybrid Mapping server at Whitehead Institute/MIT Center for Genome Research for analysis. The result demonstrated that ELF5 is localised to chromosome 11. The markers most tightly linked to ELF5 were D11S3990 (6.5cR) and D11S3998 (15.9 cR) (lod score > 3.0), and these markers are located in the region of 11p13-15 (Figure 3). This chromosomal region frequently undergoes loss of heterozygosity (LOH) in several types of carcinoma (Baffa *et al.*, 1996; Dahiya *et al.*, 1997; Hirose *et al.*, 1996; Iizuka *et al.*, 1995; Kawana *et al.*, 1997; Lichy *et al.*, 1998; Wilson *et al.*, 1996).

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#### EXAMPLE 4

##### EXPRESSION PATTERN OF ELF5 IN MOUSE TISSUES

Poly(A)<sup>+</sup> mRNA material derived from various mouse tissues were analysed by Northern  
5 blot hybridization using the murine ELF5 cDNA as a probe. A GAPDH probe was then  
used to control for RNA loading.

Analysis of ELF5 expression in adult mouse tissues revealed that ELF5 has a restricted  
expression pattern. Expression of two ELF5 transcripts, ELF5-a (2.5 kb) and ELF5-b  
10 (1.5 kb), were observed in lung (Lu), kidney (Ki), stomach (St), ovary (Ov), tongue  
(To), bladder (Bl), and day 2 pregnant (2 Ma) and day 10 pregnant (10 Ma) mammary  
glands, but no expression was observed in liver (Li), heart (He), small intestine (Sm),  
spleen (Sp), thymus (Th), pancreas (Pa), skeletal muscle (Sk), colon (Co) or fat (2 Fa  
and 10 Fa) (Figure 4a). Fat from day 2 (2 Fa) and day 10 (10 Fa) pregnant mice was  
15 used as a control for mammary expression, since the mammary gland contains much fat  
tissue. A single transcript was observed in brain (arrow - approximately 2.1 kb), but of  
a different size to either of the two ELF5 transcripts in other organs.

The expression of ELF5 was examined in the neonatal mouse (Figure 4b) and during  
20 embryogenesis on days 19, 17 and 16 (Figure 4c), and observed a similar expression  
pattern compared to that of the adult. However, at day 16 stage of embryogenesis low  
levels of ELF5 expression were detected in brain (regular sized transcripts) and small  
intestine, in addition to the expression pattern observed in the adult.

25 Placental expression of ELF5 displayed an interesting pattern during stages of  
embryogenesis (Figure 4d). Both transcripts were increasingly expressed from day 9.5 to  
day 13 before an overall decrease observed from day 14 to day 19, although some  
expression was observed at day 17.

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The two predominant ELF5 mRNA transcripts were observed in variable ratios in different tissues, suggesting that polyadenylation sites may be utilized differentially, or the two transcripts are subject to differential degradation. ELF5-a was expressed more strongly in neonatal and embryonic lung and kidney (Figures 4b and c), and adult ovary (Figure 4a), compared to ELF5-b. Conversely, ELF5-b was stronger in adult tongue (Figure 4a), and in all developmental stages of stomach (Figures 4a, b and c), compared to ELF5-a. In some RNA samples a further large (> 10 kb) transcript was variably observed.

10

### EXAMPLE 5

#### EXPRESSION PATTERN OF ELF5 IN HUMAN TISSUES AND CANCER CELL LINES

Expression of ELF5 in adult human organs was also analysed by Northern blot of poly(A)<sup>+</sup> mRNA probed with the human ELF5 cDNA (Figure 5a). A single transcript of approximately 2.5 kb was strongly expressed in kidney (Ki) and prostate (Pr). However, much longer exposures of blots demonstrated just detectable expression of ELF5 in placenta (Pl) and lung (Lu). Further, ELF5 was cloned from human lung and placenta cDNA libraries, confirming that it is expressed in these tissues, albeit probably at very low levels.

ELF5 expression in human cancers was examined. A panel of cancer cell lines, including carcinomas of the ovary (CaOv-3), breast (BT-549, ZR-75-1, T47D), kidney (786-0), liver (SK-HEP-1), lung (A549), amnion (WISH), prostate (DU145, PC3) and endometrium (HEC-1), and melanoma (MEL28), T-cell leukemia (Jurkat) and erythroid leukemia (K562), were analysed for ELF5 expression by RNase protection assay (Figure 5b). A primary fibroblast cell line (CCL32SK) was also included as a sample of non-transformed cells. Of all these cell lines only T47D, a progesterone sensitive ductal breast carcinoma, was observed to express ELF5.

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To evaluate the possibility that lack of ELF5 expression in carcinoma was due to genomic alterations, a panel of breast and lung carcinoma derived cell lines were analysed by Southern blot (Figure 5c). ELF5 gene dosage was compared to that present in DNA from normal human blood (based on the 6.5 kb *Bgl*II fragment) and controlled by hybridization with a  $\beta$ -actin cDNA probe. These results are summarized in the lower panel, where '2' represents a normal allele complement. No evidence was found for allelic loss or gene rearrangement in the two breast carcinoma cell lines that did not express ELF5 (BT-549 - lane 2, ZR-75-1 - lane 3). However, of nine lung carcinoma cell lines, evidence for loss of an ELF5 allele was observed in two (NCI-H358 - lane 8, NCI-H441 - lane 11). Hybridization with an ELF3 cDNA probe, which is localised to the long arm of chromosome 1 (Tymms *et al.*, 1997), helped to confirm the specific loss of ELF5 alleles. Two other lung carcinoma lines (SK-LU-1 - lane 10, NCI-H661 - lane 13) displayed hybridization with multiple fragments (shaded arrows) in addition to those observed in normal DNA (solid arrows), possibly indicating that at least one ELF5 allele has been rearranged in these lines. Confirmation of rearrangement, rather than restriction fragment length polymorphism (RFLP), was made by additional restriction digests. Some cell lines appeared to have amplification or additional copies of the ELF5 gene. One of these, T47D (lane 4), was the only cell line demonstrated to express ELF5, and another, SK-LU-1 (lane 10), appeared to have rearranged alleles.

20

## EXAMPLE 6

### SEQUENCE-SPECIFIC BINDING OF ELF5 TO DNA SEQUENCES CONTAINING CONSENSUS ETS SITES

Although ELF5 displays similarity to the consensus ETS domain, characterising it as an ETS family member, this sequence is still quite divergent from most other ETS family members. The hallmark of ETS factors to bind DNA sites containing a GGAA-core in a sequence-specific manner is however shared by ELF5, demonstrating an additional functional similarity to the ETS family. A recombinant ELF5 HIS-tag protein of approximately 29 kD, expressed in *E. coli* and purified by metal-affinity chromatography

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(Figure 6a, lane 4), displayed strong binding to consensus ETS binding sites, as analysed by electrophoretic mobility shift assay (EMSA) (Figure 6b). ELF5 bound the E74 oligonucleotide (containing a GGAA-core) (lane 1), but not to the E74ml oligonucleotide (which had been mutated to an AGAA-core) (lane 2). The first G-residue of the core has  
 5 been demonstrated to be a physical point of DNA contact for ETS1, and consequently essential for DNA binding (Fisher *et al.*, 1991; Nye *et al.*, 1992). Thus, ELF5 displays sequence specific binding to a consensus ETS binding site, binding that is disrupted by a mutation known to similarly affect other ETS family members. These results were confirmed through competition analysis. The ELF5-E74 complex (lane 3) was efficiently  
 10 competed by the addition of a 100-fold excess of unlabeled E74 (lane 4), but not by E74ml (lane 5).

ELF5 also displayed sequence specific binding to different consensus ETS binding sequences, and did so with differential affinity (Figure 6b). Competition of the ELF5-  
 15 E74 complex (lane 3) was achieved by consensus ETS sites from the GM-CSF promoter (lane 6), *erb*-B2 promoter (lane 7) and moloney sarcoma virus (MSV) long terminal repeat (LTR) (lane 8). The relative ability of ELF5 to bind these sequences occurred in the order: E74 > *erb*B2 > MSV > GM-CSF. ELF5 did not appear to be competed at all by an oligonucleotide containing a consensus AP1 binding site (lane 9). ETS1 binding to  
 20 E74 was used as a positive control (lane 10).

## EXAMPLE 7

### MOUSE ELF5 ACTS AS A TRANSCRIPTIONAL ACTIVATOR

25 In addition to DNA binding, another characteristic of most ETS factors is their ability to transactivate from binding sites in promoters and enhancers.

A reporter construct, containing the chloramphenicol acetyl-transferase (CAT) driven by a minimal TK promoter and multiple ETS/API binding sites (from the polyomavirus  
 30 enhancer), was co-transfected into COS cells together with an ELF5 expression construct

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(Figure 7). Analysis of CAT activities revealed that ELF5 expression resulted in an average five-fold transactivation of the reporter. Further, this transactivation was inhibited by addition of an anti-sense ELF5 mRNA expression vector, indicating that ELF5 transactivation was due specifically to the product translated from the sense  
5 construct.

### EXAMPLE 8

#### ISOLATION AND CHARACTERIZATION OF FULL-LENGTH MURINE ELF5 CDNA

10

The murine *Elf5* cDNA was isolated from an adult lung cDNA library in Lambda ZAPII (Stratagene) following screening with a cDNA probe containing the ETS domain region of human *ELF3*. Additional 5' sequence and 3' sequence were obtained by RT-PCR using a Marathon cDNA synthesis Kit (Clontech) and RACE (Rapid Amplification using day 14  
15 murine of cDNA Ends) placental Poly(A)<sup>+</sup> RNA. The murine *Elf5*-specific PCR products were cloned into pGEM-T vector (Promega Corp., Madison, WI, USA). All cDNA sequences were confirmed by sequencing both strands at least once. 5'-RACE gene-specific primer 1: 5'-GCCAGTCTTG-GTCTCTTCAGCATC-3' (SEQ ID NO:8); 5'-RACE nested-gene-specific primer 2: 5'-AGGAGATGCAGTTGGCATCAAGCT-3' (SEQ ID NO:9); 3'-  
20 RACE gene-specific primer 1: 5'-AGCCAGTGTTATGGGTGCTG-3' (SEQ ID NO:10); 3'-RACE nested-gene-specific primer 2: 5'-ACAGTCACTTGATCCACGGCCAATCC-3' (SEQ ID NO:11).

### EXAMPLE 9

25

#### ISOLATION OF HUMAN ELF5 CODING SEQUENCE

A human *ELF5* cDNA fragment was isolated from a human lung cDNA library (GIBCO BRL) following screening with a cDNA probe containing the coding sequence of mouse *Elf5*. The coding sequence was then obtained by RT-PCR using a Marathon cDNA  
30 synthesis Kit (Clontech) and RACE (Rapid Amplification of cDNA Ends) using human placental Poly(A)<sup>+</sup> RNA. The human *ELF5*-specific PCR products were cloned into



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pGEM-T vector (Promega Corp., Madison, WI, USA). All cDNA sequences were confirmed by sequencing both strands at least once.

### EXAMPLE 10

#### STS CONTENT MAPPING

The following sequence specific primers for human ELF5 were used for PCR. Forward primer: 5'-CCTGTGACTCATACTGGACATC-3' (SEQ ID NO:12); Reverse primer: 5'-CTTGTGTGCGGATGTTCTGG-3' (SEQ ID NO:13). The PCR reactions were performed in Opti-Primer™ 10 x buffer #3 (100 mM Tris-HCl pH 8.3, 35 mM MgCl<sub>2</sub>, 250 mM KCl) with 1 µl of Master Mix 50 x buffer (20 mM Tris-HCl pH 8.0, 250 nM EDTA) (Opti-Primer™ PCR Optimization Kit, Stratagene), 50 ng of template DNA, 0.2 µg of each primer, 1 µl of 10 mM dNTPs and 0.25 U of Taq DNA polymerase in a total volume of 50 µl. PCR parameters were an initial denaturation step at 94°C for 1 min, followed by 30 cycles of 94°C (1 min), 60°C (1 min), 72°C (1 min). For Genebridge 4 Radiation Hybrid DNA panel (UK GHMP Resource Centre), PCR reactions were performed separately for each of the individual hybrids. The PCR results from the 93 hybrids were submitted to the Radiation Hybrid Mapping server at Whitehead Institute/MIT Center for Genome Research (<http://www.genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>). The STS content mapping experiment was performed in duplicate and included PCR reactions with no DNA, total human DNA and total hamster DNA as controls.

### EXAMPLE 11

#### SOUTHERN AND NORTHERN BLOT ANALYSIS

Northern analysis of *ELF5* expression in human adult organs was performed with commercially available blots containing 2 µg of Poly(A)<sup>+</sup> RNA (Clontech). For other Northern blots POLY(A)<sup>+</sup> mRNA was isolated by a modification of Gonda *et al.* (1992). Genomic DNA was isolated by standard techniques (Sambrook *et al.*, 1997). Random-primed probes using a 898 bp human *ELF5* cDNA fragment and a 940 bp *StyI* mouse *Elf5* cDNA fragment were generated and Southern/Northern hybridizations performed using

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standard procedures. Blots were re-probed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or  $\beta$ -actin cDNAs to verify RNA/DNA loading.

## EXAMPLE 12

5

### RNASE PROTECTION ANALYSIS

*ELF5* mRNA abundance in total RNA from human cell lines was determined as described previously (Tymms, 1995). Anti-sense RNA probes for human *ELF5* and GAPDH transcribed from linearized plasmid vectors generated full-length probes of 388 bp and 216 bp, respectively. The protected products generated by hybridization and RNase digestion are 298 bp for *ELF5* and 150 bp for GAPDH.

## EXAMPLE 13

### CELL LINES AND CULTURE

15

Monkey COS7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin, and maintained in a humidified incubator at 5% CO<sub>2</sub> and 37°C.

20

## EXAMPLE 14

### PLASMIDS

pHis6-Elf5 expression vector was made as follows: The murine *Elf5* cDNA was amplified using PCR oligonucleotide primers (5'-CGGGATCCTTGGACTCCGTAACCCATAGC-3' (SEQ ID NO:14) and 5'-GCAGATCTCAGAGTTTCTCTTCCTGCC-3' (SEQ ID NO:15)) containing a *Bam*HI restriction site followed by 21 nucleotides of the murine *Elf5* coding sequence and a *Bgl*II restriction site followed by 19 nucleotides complementary to the last 20 nucleotides of the *Elf5* coding sequence. The PCR fragment was cloned into the pGEM-T vector (Promega Corp., Madison, WI, USA), the *Bam*HI-*Sac*I restriction fragment with the *Elf5* coding sequence was then cloned into the *Bam*HI-*Sac*I sites of the pQE30 (Qiagen, Inc. Chatsworth, CA, USA) bacterial expression vector resulting in a N-terminal fusion of

*Elf5* protein to six histidine residues (His-Tag).

The *Elf5* mammalian expression construct (pBOSElf5s) contains the full mouse *Elf5* cDNA blunt cloned into the T4 polymerase blunted *Xba*I site of pEFBOS (Mizushima and Natata, 1990). Expression from pEFBOS is driven by the elongation factor-1 promoter. The *Elf5* anti-sense expression construct is similar, but with reverse orientation of the *Elf5* polyomavirus enhancer oligonucleotides into the *Bam*HI site of pBLCAT2.

## EXAMPLE 15

### 10 ELECTROPHORETIC MOBILITY SHIFT ASSAYS (EMSA)

Purified recombinant *Elf5* and Ets1 proteins were produced as 6XHis-tag fusions in *E. coli* using the QIAexpress expression system (Qiagen). Overnight cultures were diluted 1/10 in LB broth and grown for 1 h at 37°C. Expression of recombinant proteins were induced by 15 addition of 0.1 mM IPTG and culture of cells for 2 h. Cells were harvested and sonicated in lysis buffer (6 M guanidine, 20 mM Tris-HCl, 50 mM NaCl, pH 8.0), and cell debris removed by centrifugation. One ml of metal His-affinity resin was incubated with supernatants for 30 min, collected, washed in wash buffer (8 M urea, 20 mM Tris-HCl, 50 mM NaCl, pH 8.0), and resuspended in renaturation buffer (20 mM Tris-HCl, 50 mM NaCl, 20 3 mM dithiothreitol (DTT), pH 8.0). Proteins were eluted from the beads in renaturation buffer supplemented with 100 mM imidazole. Purification and integrity of recombinant proteins were confirmed by denaturing SDS-polyacrylamide gel electrophoresis (PAGE).

DNA binding experiments with recombinant proteins were performed using EMSA, as 25 previously described (Thomas *et al*, 1995, 1997). Briefly, purified double stranded oligonucleotides were labeled with  $\gamma$ -<sup>32</sup>P dATP and T4 polynucleotide kinase. Oligonucleotide probe (1 ng) was incubated for 10 min with approximately 20 ng purified *Elf5/Ets1* protein in DNA binding buffer (1 mM EDTA, 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 3 mM DTT, 1 mg/ml BSA, 500 ng/ml poly-d(I-C)d(I-C), 500 ng/ml poly dI-dC, 200 30 ng/ml sheared salmon sperm DNA),  $\pm$  100 ng unlabelled competitor oligonucleotides, in 10  $\mu$ l final volume. Assays were run through non-denaturing, 7% acrylamide (29 acrylamide:1

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bis-acrylamide), 0.5 x TBE gels at 4°C.

#### **EXAMPLE 16**

##### **LACK OF ELF5 EXPRESSION IN HUMAN PRIMARY BREAST CARCINOMAS**

5

A panel of human primary breast carcinoma samples were analysed for ELF5 expression by *in situ* hybridization (Hogan *et al.*, 1994). Section from parafin-embedded samples were hybridized with ELF5 <sup>33</sup>P-labelled antisense RNA, and signals were detected with a photosensitive emulsion. Serial sections were also stained with eosin and haematoxylin.

10

A preliminary examination of ELF5 expression shows that ELF5 is not detectable in 20 out of 20 human breast carcinomas studied, whereas it is strongly expressed in adjacent normal epithelium and in epithelial cells from normal subjects (Figure 8).

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 898 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 110..875

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CAAGGCTACA GGTGTCTTTA TTTCCACTGC ACGCTGGTGC TGGGAGCGCC TGCCTTCTCT      60
TGCCTTGAAA GCCTCCTCTT TGGACCTAGC CACCGCTGCC CTCACGGTA ATG TTG      115
                               Met Leu
                               1

GAC TCG GTG ACA CAC AGC ACC TTC CTG CCT AAT GCA TCC TTC TGC GAT      163
Asp Ser Val 5 Thr His Ser Thr 10 Leu Pro Asn Ala 15 Phe Cys Asp

CCC CTG ATG TCG TGG ACT GAT CTG TTC AGC AAT GAA GAG TAC TAC CCT      211
Pro Leu Met Ser Trp Thr Asp 25 Leu Phe Ser Asn Glu Glu Tyr Tyr Pro

GCC TTT GAG CAT CAG ACA GCC TGT GAC TCA TAC TGG ACA TCA GTC CAC      259
Ala Phe Glu His Gln Thr 40 Ala Cys Asp Ser Tyr 45 Trp Thr Ser Val His 50

CCT GAA TAC TGG ACT AAG CGC CAT GTG TGG GAG TGG CTC CAG TTC TGC      307
Pro Glu Tyr Trp 55 Thr Lys Arg His Val 60 Trp Glu Trp Leu Gln Phe Cys 65

TGC GAC CAG TAC AAG TTG GAC ACC AAT TGC ATC TCC TTC TGC AAC TTC      355
Cys Asp Gln Tyr 70 Lys Leu Asp Thr 75 Asn Cys Ile Ser Phe 80 Cys Asn Phe

AAC ATC AGT GGC CTG CAG CTG TGC AGC ATG ACA CAG GAG GAG TTC GTC      403
Asn Ile Ser 85 Gly Leu Gln Leu 90 Cys Ser Met Thr Gln 95 Glu Glu Phe Val

GAG GCA GCT GGC CTC TGC GGC GAG TAC CTG TAC TTC ATC CTC CAG AAC      451
Glu Ala Ala Gly Leu Cys 105 Gly Glu Tyr Leu Tyr 110 Phe Ile Leu Gln Asn

ATC CGC ACA CAA GGT TAC TCC TTT TTT AAT GAC GCT GAA GAA AGC AAG      499
Ile Arg Thr Gln Gly Tyr 120 Ser Phe Phe Asn Asp Ala Glu Glu Ser Lys 130

GCC ACC ATC AAA GAC TAT GCT GAT TCC AAC TGC TTG AAA ACA AGT GGC      547
Ala Thr Ile Lys Asp 135 Tyr Ala Asp Ser 140 Asn Cys Leu Lys Thr Ser Gly

ATC AAA AGT CAA GAC TGT CAC AGT CAT AGT AGA ACA AGC CTC CAA AGT      595
Ile Lys Ser Gln Asp Cys His Ser 155 His Ser Arg Thr Ser 160 Leu Gln Ser

TCT CAT CTA TGG GAA TTT GTA CGA GAC CTG CTT CTA TCT CCT GAA GAA      643
Ser His Leu Trp Glu Phe Val 170 Arg Asp Leu Leu Leu 175 Ser Pro Glu Glu

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AAC TGT GGC ATT CTG GAA TGG GAA GAT AGG GAA CAA GGA ATT TTT CGG	691
Asn Cys Gly Ile Leu Glu Trp Glu Asp Arg Glu Gln Gly Ile Phe Arg	
180 185 190	
GTG GTT AAA TCG GAA GCC CTG GCA AAG ATG TGG GGA CAA AGG AAG AAA	739
Val Val Lys Ser Glu Ala Leu Ala Lys Met Trp Gly Gln Arg Lys Lys	
195 200 205 210	
AAT GAC AGA ATG ACA TAT GAA AAG TTG AGC AGA GCC CTG AGA TAC TAC	787
Asn Asp Arg Met Thr Tyr Glu Lys Leu Ser Arg Ala Leu Arg Tyr Tyr	
215 220 225	
TAT AAA ACA GGA ATT TTG GAG CGG GTT GAC CGA AGG TTA GTG TAC AAA	835
Tyr Lys Thr Gly Ile Leu Glu Arg Val Asp Arg Arg Leu Val Tyr Lys	
230 235 240	
TTT GGA AAA AAT GCA CAC GGG TGG CAG GAA GAC AAG CTA T GATCTGCTCC	885
Phe Gly Lys Asn Ala His Gly Trp Gln Glu Asp Lys Leu	
245 250 255	
AGGCATCAAG CTC	898

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 255 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Leu	Asp	Ser	Val	Thr	His	Ser	Thr	Phe	Leu	Pro	Asn	Ala	Ser	Phe
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Cys	Asp	Pro	Leu	Met	Ser	Trp	Thr	Asp	Leu	Phe	Ser	Asn	Glu	Glu	Tyr
	20							25					30		
Tyr	Pro	Ala	Phe	Glu	His	Gln	Thr	Ala	Cys	Asp	Ser	Tyr	Trp	Thr	Ser
	35						40					45			
Val	His	Pro	Glu	Tyr	Trp	Thr	Lys	Arg	His	Val	Trp	Glu	Trp	Leu	Gln
	50					55					60				
Phe	Cys	Cys	Asp	Gln	Tyr	Lys	Leu	Asp	Thr	Asn	Cys	Ile	Ser	Phe	Cys
	65				70					75					80
Asn	Phe	Asn	Ile	Ser	Gly	Leu	Gln	Leu	Cys	Ser	Met	Thr	Gln	Glu	Glu
			85						90					95	
Phe	Val	Glu	Ala	Ala	Gly	Leu	Cys	Gly	Glu	Tyr	Leu	Tyr	Phe	Ile	Leu
			100					105					110		
Gln	Asn	Ile	Arg	Thr	Gln	Gly	Tyr	Ser	Phe	Phe	Asn	Asp	Ala	Glu	Glu
	115					120						125			
Ser	Lys	Ala	Thr	Ile	Lys	Asp	Tyr	Ala	Asp	Ser	Asn	Cys	Leu	Lys	Thr
	130				135						140				
Ser	Gly	Ile	Lys	Ser	Gln	Asp	Cys	His	Ser	His	Ser	Arg	Thr	Ser	Leu
145					150				155						160

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Gln	Ser	Ser	His	Leu 165	Trp	Glu	Phe	Val	Arg 170	Asp	Leu	Leu	Leu	Ser 175	Pro
Glu	Glu	Asn	Cys 180	Gly	Ile	Leu	Glu	Trp 185	Glu	Asp	Arg	Glu	Gln 190	Gly	Ile
Phe	Arg	Val 195	Val	Lys	Ser	Glu	Ala 200	Leu	Ala	Lys	Met	Trp 205	Gly	Gln	Arg
Lys	Lys 210	Asn	Asp	Arg	Met	Thr 215	Tyr	Glu	Lys	Leu	Ser 220	Arg	Ala	Leu	Arg
Tyr 225	Tyr	Tyr	Lys	Thr	Gly 230	Ile	Leu	Glu	Arg	Val 235	Asp	Arg	Arg	Leu	Val 240
Tyr	Lys	Phe	Gly	Lys 245	Asn	Ala	His	Gly	Trp 250	Gln	Glu	Asp	Lys	Leu 255	

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 637 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 134..614

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TGCCCTCACG	GTA	ATG	TTG	GAC	TCG	GTG	ACA	CAC	AGC	ACC	TTC	CTG	CCT		169	
	Met	Leu	Asp	Ser	Val	Thr	His	Ser	Thr	Phe	Leu	Pro				
	1					5					10					
AAT	GCA	TCC	CTC	TGC	GAT	CCC	CTG	ATG	TCG	TGG	ACT	GAT	CTG	TTC	AGC	217
Asn	Ala	Ser	Leu	Cys	Asp	Pro	Leu	Met	Ser	Trp	Thr	Asp	Leu	Phe	Ser	
	15						20					25				
AAT	GAA	GAG	TAC	TAC	CCT	GCC	TTT	GAG	CAT	CAG	ACA	GAT	GCT	GAT	TCC	265
Asn	Glu	Glu	Tyr	Tyr	Pro	Ala	Phe	Glu	His	Gln	Thr	Asp	Ala	Asp	Ser	
	30					35					40					
AAC	TGC	TTG	AAA	ACA	AGT	GGC	ATC	AAA	AGC	CAA	GAC	TGT	CAC	AGT	CAT	313
Asn	Cys	Leu	Lys	Thr	Ser	Gly	Ile	Lys	Ser	Gln	Asp	Cys	His	Ser	His	
	45				50				55						60	
AGT	AGA	ACA	AGC	CTC	CAA	AGT	TCT	CAT	CTA	TGG	GAA	TTT	GTA	CGA	GAC	361
Ser	Arg	Thr	Ser	Leu	Gln	Ser	Ser	His	Leu	Trp	Glu	Phe	Val	Arg	Asp	
				65					70				75			
CTG	CTT	CTA	TCT	CCT	GAA	GAA	AAC	TGT	GGC	ATT	CTG	GAA	TGG	GAA	GAT	409
Leu	Leu	Leu	Ser	Pro	Glu	Glu	Asn	Cys	Gly	Ile	Leu	Glu	Trp	Glu	Asp	
			80					85					90			

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AGG GAA CAA GGA ATT TTT CGG GTG GTT AAA TCG GAA GCC CTG GCA AAG	457
Arg Glu Gln Gly Ile Phe Arg Val Val Lys Ser Glu Ala Leu Ala Lys	
95 100 105	
ATG TGG GGA CAA AGG AAG AAA AAT GAC AGA ATG ACA TAT GAA AAG TTG	505
Met Trp Gly Gln Arg Lys Lys Asn Asp Arg Met Thr Tyr Glu Lys Leu	
110 115 120	
AGC AGA GCC CTG AGA TAC TAC TAT AAA ACA GGA ATT TTG GAG CGG GTT	553
Ser Arg Ala Leu Arg Tyr Tyr Lys Thr Gly Ile Leu Glu Arg Val	
125 130 135 140	
GAC CGA AGG TTA GTG TAC AAA TTT GGA AAA AAT GCA CAC GGG TGG CAG	601
Asp Arg Arg Leu Val Tyr Lys Phe Gly Lys Asn Ala His Gly Trp Gln	
145 150 155	
GAA GAC AAG CTA T GATCTGCTCC AGGCATCAAG CTC	637
Glu Asp Lys Leu	
160	

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 160 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Leu	Asp	Ser	Val	Thr	His	Ser	Thr	Phe	Leu	Pro	Asn	Ala	Ser	Leu
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Cys	Asp	Pro	Leu	Met	Ser	Trp	Thr	Asp	Leu	Phe	Ser	Asn	Glu	Glu	Tyr
			20					25					30		
Tyr	Pro	Ala	Phe	Glu	His	Gln	Thr	Asp	Ala	Asp	Ser	Asn	Cys	Leu	Lys
		35					40					45			
Thr	Ser	Gly	Ile	Lys	Ser	Gln	Asp	Cys	His	Ser	His	Ser	Arg	Thr	Ser
	50					55					60				
Leu	Gln	Ser	Ser	His	Leu	Trp	Glu	Phe	Val	Arg	Asp	Leu	Leu	Leu	Ser
65					70					75					80
Pro	Glu	Glu	Asn	Cys	Gly	Ile	Leu	Glu	Trp	Glu	Asp	Arg	Glu	Gln	Gly
			85						90					95	
Ile	Phe	Arg	Val	Val	Lys	Ser	Glu	Ala	Leu	Ala	Lys	Met	Trp	Gly	Gln
			100					105					110		
Arg	Lys	Lys	Asn	Asp	Arg	Met	Thr	Tyr	Glu	Lys	Leu	Ser	Arg	Ala	Leu
		115					120					125			
Arg	Tyr	Tyr	Tyr	Lys	Thr	Gly	Ile	Leu	Glu	Arg	Val	Asp	Arg	Arg	Leu
	130					135					140				
Val	Tyr	Lys	Phe	Gly	Lys	Asn	Ala	His	Gly	Trp	Gln	Glu	Asp	Lys	Leu
145					150					155					160

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## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2224 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 117..876

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATG TTG GAC TCC GTA ACC CAT AGC ACC TTC CTG CCC AAC GCA TCC TTC Met Leu Asp Ser Val Thr His Ser Thr Phe Leu Pro Asn Ala Ser Phe 1 5 10 15	164
TGT GAC CCC CTG ATG CCT TGG ACC GAT CTG TTC AGC AAT GAA GAC TAC Cys Asp Pro Leu Met Pro Trp Thr Asp Leu Phe Ser Asn Glu Asp Tyr 20 25 30	212
TAC CCT GCC TTT GAG CAT CAG ACA GCC TGT GAT TCC TAC TGG ACA TCA Tyr Pro Ala Phe Glu His Gln Thr Ala Cys Asp Ser Tyr Trp Thr Ser 35 40 45	260
GTG CAC CCT GAA TAC TGG ACC AAG CGC CAT GTC TGG GAA TGG CTC CAA Val His Pro Glu Tyr Trp Thr Lys Arg His Val Trp Glu Trp Leu Gln 50 55 60	308
TTC TGC TGT GAC CAG TAC AAG CTT GAT GCC AAC TGC ATC TCC TTC TGT Phe Cys Cys Asp Gln Tyr Lys Leu Asp Ala Asn Cys Ile Ser Phe Cys 65 70 75 80	356
CAC TTC AAC ATC AGC GGC CTG CAG CTC TGC AGC ATG ACG CAG GAG GAG His Phe Asn Ile Ser Gly Leu Gln Leu Cys Ser Met Thr Gln Glu Glu 85 90 95	404
TTC ATT GAG GCA GCC GGC ATC TGT GGG GAG TAC CTG TAC TTC ATT CTC Phe Ile Glu Ala Ala Gly Ile Cys Gly Glu Tyr Leu Tyr Phe Ile Leu 100 105 110	452
CAG AAC ATT CGC TCG CAA GGT TAC TCC TTT TTC AAT GAT GCT GAA GAG Gln Asn Ile Arg Ser Gln Gly Tyr Ser Phe Phe Asn Asp Ala Glu Glu 115 120 125	500
ACC AAG ACT GGC ATC AAA GAC TAT GCT GAT TCC AGT TGC TTG AAA ACA Thr Lys Thr Gly Ile Lys Asp Tyr Ala Asp Ser Ser Cys Leu Lys Thr 130 135 140	548
AGT GGC ATC AAG AGT CAA GAC TGT CAC AGC CGA ACA AGC CTC CAA AGT Ser Gly Ile Lys Ser Gln Asp Cys His Ser Arg Thr Ser Leu Gln Ser 145 150 155 160	596
TCT CAC CTG TGG GAA TTT GTC AGA GAC TTG CTG CTG TCC CCT GAA GAG Ser His Leu Trp Glu Phe Val Arg Asp Leu Leu Leu Ser Pro Glu Glu 165 170 175	644

- 60 -

AAC TGT GGC ATC CTG GAA TGG GAA GAC AGG GAG CAG GGC ATT TTC CGA Asn Cys Gly Ile Leu Glu Trp Glu Asp Arg Glu Gln Gly Ile Phe Arg 180 185 190	692
GTG GTT AAG TCA GAA GCC CTG GCA AAG ATG TGG GGA CAA AGG AAG AAG Val Val Lys Ser Glu Ala Leu Ala Lys Met Trp Gly Gln Arg Lys Lys 195 200 205	740
AAT GAC AGG ATG ACG TAC GAG AAG CTG AGC CGA GCC CTG AGA TAC TAC Asn Asp Arg Met Thr Tyr Glu Lys Leu Ser Arg Ala Leu Arg Tyr Tyr 210 215 220	788
TAT AAA ACG AGA ATT CTG GAG CGG GTT GAC CGG AGG TTA GTG TAC AAA Tyr Lys Thr Arg Ile Leu Glu Arg Val Asp Arg Arg Leu Val Tyr Lys 225 230 235 240	836
TTT GGA AAG AAC GCG CAC GGG TGG CAG GAA GAG AAA CTC T GATGGACACC Phe Gly Lys Asn Ala His Gly Trp Gln Glu Glu Lys Leu 245 250	886
GGACACCAGG CTCATTTGAT GGATTTCTGT TGTGTGAAAC AATCAGATCA AACTAGACAT	946
TTGAAAGTCT CCCTCCTCCT CCTCCTCCCC CTCCTTCCCC TCCTCTTCTT CCTCCCCCTC	1006
CTCCTCTTCA AAACCTACAA ACACACTGAT AAAATTTCTG CATGTCTCAG CTTACATTTG	1066
AATTCAGTTG TTGTCTATTG GGGCGATGCC ATCAGCCCTT AAGCAATCGT CTTCATCCCCA	1126
AGGGGGAGGA AGGGATGGTC TTGTGGCAAC TTGGTGTGAC ACTGTCTCCT TAATGAAGTG	1186
TTTGGAGCTA AGGGAGCCAG TGTTATGGGT GCTGTTTCAC AAGAGGACCC GTTGCACCAT	1246
TAAGACACAT GATCCTCCCG TTCCAGGGGT TCTGAGCGGT CGACTGAGGC AGCTTGCCTG	1306
TGGTTAGTTT TTAGGAAAGG GAGATGTAAG ACTTCCTTGC TTTAGATTTG AAATTATCAC	1366
AGTTATATTC CATAGAAGAA TTTTAAATTA AAAAAATTTT AGTGGCTAAG CCACTAAACT	1426
GGGACCTAAT TGGATGTAGC CTAAGTTACT AATAAGTTCT TAACCAGATC ACCATTTCCA	1486
ACCACTTAGC CACAGTCACT TGATCCACGG CCAATCCTTC TGAACCTAAC ATCCTTGTAG	1546
TTAGTCACCT TGGGAATTGC TACCTAGATT GTTACCCCTT TCACCTCACT GGTGGCTATC	1606
ATCAGGTCTA CAGTGACCTG ATCAACAGAC ATGTGCATTA ATTTCTAAAT CACTGCTGTG	1666
CCTATGATTC AAACCGTCAG CGTGTTTCAGT TTATTGATTC TCTCTGAGGT CGGAATTTAT	1726
TGATTCTCTC TGAGGCTAAG ACATTAAACC TTTACCAAGC AGAGAACGTC CTAACAAGCC	1786
ACGATAGCCG AACACAGCAT CGATCTCTTC TCTTTTCTGA TGAATACTCA AACTTTCCAA	1846
CATATTCTCT TCACAAAAGT AAAGACAGTG AATTTACATC AATCAACGTT CATGGGTTAA	1906
AGTCTGCACT GACATTTCTT TGTCTGCCGT TGCATGCCGT TGGCATGCAA GGTGTTAATG	1966
ACCTGCAACA TGGTGGAGTG CCCTGAACCC TAACTTCCCC AGAGTTGGGA CTGTCTAGTG	2026
ACCGGCACTG AATAGCAATG CAGGCTGAAG ACCTCCAGGT TTAGAATTTA ACCTCAAAAG	2086
TAACTTGTTT TAAAAAAGAA ATGTGAATTA CTGTAAATA ATCTATTTT GGATTCGTGT	2146
GTTTTTCAGG TGGATATAGT TTATAAACAA TGTGAATAAA AAATATTTAA CATGTTTAAA	2206
AAAAAAAAAA AAAAAAAA	2224

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## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1528 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 117..876

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATCACACGGC TACAGGTGCC TTTATTTCTA CAGTCCGCTG GTGCTGGGAG CGCGCTTGCC	60
TTCTCTTGCC TTGAAAGCCT TCTGTCTGGA CCTAGCCACC ACTTGTCTTC ACGGTG	116
ATG TTG GAC TCC GTA ACC CAT AGC ACC TTC CTG CCC AAC GCA TCC TTC Met Leu Asp Ser Val Thr His Ser Thr Phe Leu Pro Asn Ala Ser Phe 1 5 10 15	164
TGT GAC CCC CTG ATG CCT TGG ACC GAT CTG TTC AGC AAT GAA GAC TAC Cys Asp Pro Leu Met Pro Trp Thr Asp Leu Phe Ser Asn Glu Asp Tyr 20 25 30	212
TAC CCT GCC TTT GAG CAT CAG ACA GCC TGT GAT TCC TAC TGG ACA TCA Tyr Pro Ala Phe Glu His Gln Thr Ala Cys Asp Ser Tyr Trp Thr Ser 35 40 45	260
GTG CAC CCT GAA TAC TGG ACC AAG CGC CAT GTC TGG GAA TGG CTC CAA Val His Pro Glu Tyr Trp Thr Lys Arg His Val Trp Glu Trp Leu Gln 50 55 60	308
TTC TGC TGT GAC CAG TAC AAG CTT GAT GCC AAC TGC ATC TCC TTC TGT Phe Cys Cys Asp Gln Tyr Lys Leu Asp Ala Asn Cys Ile Ser Phe Cys 65 70 75 80	356
CAC TTC AAC ATC AGC GGC CTG CAG CTC TGC AGC ATG ACG CAG GAG GAG His Phe Asn Ile Ser Gly Leu Gln Leu Cys Ser Met Thr Gln Glu Glu 85 90 95	404
TTC ATT GAG GCA GCC GGC ATC TGT GGG GAG TAC CTG TAC TTC ATT CTC Phe Ile Glu Ala Ala Gly Ile Cys Gly Glu Tyr Leu Tyr Phe Ile Leu 100 105 110	452
CAG AAC ATT CGC TCG CAA GGT TAC TCC TTT TTC AAT GAT GCT GAA GAG Gln Asn Ile Arg Ser Gln Gly Tyr Ser Phe Phe Asn Asp Ala Glu Glu 115 120 125	500
ACC AAG ACT GGC ATC AAA GAC TAT GCT GAT TCC AGT TGC TTG AAA ACA Thr Lys Thr Gly Ile Lys Asp Tyr Ala Asp Ser Ser Cys Leu Lys Thr 130 135 140	548
AGT GGC ATC AAG AGT CAA GAC TGT CAC AGC CGA ACA AGC CTC CAA AGT Ser Gly Ile Lys Ser Gln Asp Cys His Ser Arg Thr Ser Leu Gln Ser 145 150 155 160	596
TCT CAC CTG TGG GAA TTT GTC AGA GAC TTG CTG CTG TCC CCT GAA GAG Ser His Leu Trp Glu Phe Val Arg Asp Leu Leu Leu Ser Pro Glu Glu 165 170 175	644



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AAC TGT GGC ATC CTG GAA TGG GAA GAC AGG GAG CAG GGC ATT TTC CGA	692
Asn Cys Gly Ile Leu Glu Trp Glu Asp Arg Glu Gln Gly Ile Phe Arg	
180 185 190	
GTG GTT AAG TCA GAA GCC CTG GCA AAG ATG TGG GGA CAA AGG AAG AAG	740
Val Val Lys Ser Glu Ala Leu Ala Lys Met Trp Gly Gln Arg Lys Lys	
195 200 205	
AAT GAC AGG ATG ACG TAC GAG AAG CTG AGC CGA GCC CTG AGA TAC TAC	788
Asn Asp Arg Met Thr Tyr Glu Lys Leu Ser Arg Ala Leu Arg Tyr Tyr	
210 215 220	
TAT AAA ACG AGA ATT CTG GAG CGG GTT GAC CGG AGG TTA GTG TAC AAA	836
Tyr Lys Thr Arg Ile Leu Glu Arg Val Asp Arg Arg Leu Val Tyr Lys	
225 230 235 240	
TTT GGA AAG AAC GCG CAC GGG TGG CAG GAA GAG AAA CTC T GATGGACACC	886
Phe Gly Lys Asn Ala His Gly Trp Gln Glu Glu Lys Leu	
245 250	
GGACACCAGG CTCATTGTGAT GGATTTCTGT TGTTGGAAAC AATCAGATCA AACTAGACAT	946
TTGAAAGTCT CCCTCCTCCT CCTCCTCCCC CTCCTTCCCC TCCTCTTCTT CCTCCCCCTC	1006
CTCCTCTTCA AAACCTACAA ACACACTGAT AAAATTTCTG CATGTCTCAG CTTACATTTG	1066
AATTCAGTTG TTGTCTATTG GGGCGATGCC ATCAGCCCTT AAGCAATCGT CTTCATCCCA	1126
AGGGGGAGGA AGGGATGGTC TTGTGGCAAC TTGGTGTGAC ACTGTCTCCT TAATGAAGTG	1186
TTTGGAGCTA AGGGAGCCAG TGTTATGGGT GCTGTTTCAC AAGAGGACCC GTTGCACCAT	1246
TAAGACACAT GATCCTCCCG TTCCAGGGGT TCTGAGCGGT CGACTGAGGC AGCTTGCCCTG	1306
TGGTTAGTTT TTAGGAAAGG GAGATGTAAG ACTTCCTTGC TTTAGATTTG AAATTATCAC	1366
AGTTATATTC CATAGAAGAA TTTTAAATTA AAAAAATTTT AGTGGCTAAG CCACTAAACT	1426
GGGACCTAAT TGGATGTAGC CTAAGTTACT AATAAGTTCT TAACCAGATC ACCATTTCCA	1486
ACCACTTAGC CACAGTCACT TGATCCACGG CCAATCCTTC TG	1528

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 253 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Leu Asp Ser Val Thr His Ser Thr Phe Leu Pro Asn Ala Ser Phe
1 5 10 15
Cys Asp Pro Leu Met Pro Trp Thr Asp Leu Phe Ser Asn Glu Asp Tyr
20 25 30
Tyr Pro Ala Phe Glu His Gln Thr Ala Cys Asp Ser Tyr Trp Thr Ser
35 40 45
Val His Pro Glu Tyr Trp Thr Lys Arg His Val Trp Glu Trp Leu Gln
50 55 60

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Phe Cys Cys Asp Gln Tyr Lys Leu Asp Ala Asn Cys Ile Ser Phe Cys  
 65 70 75 80  
 His Phe Asn Ile Ser Gly Leu Gln Leu Cys Ser Met Thr Gln Glu Glu  
 85 90 95  
 Phe Ile Glu Ala Ala Gly Ile Cys Gly Glu Tyr Leu Tyr Phe Ile Leu  
 100 105 110  
 Gln Asn Ile Arg Ser Gln Gly Tyr Ser Phe Phe Asn Asp Ala Glu Glu  
 115 120 125  
 Thr Lys Thr Gly Ile Lys Asp Tyr Ala Asp Ser Ser Cys Leu Lys Thr  
 130 135 140  
 Ser Gly Ile Lys Ser Gln Asp Cys His Ser Arg Thr Ser Leu Gln Ser  
 145 150 155 160  
 Ser His Leu Trp Glu Phe Val Arg Asp Leu Leu Leu Ser Pro Glu Glu  
 165 170 175  
 Asn Cys Gly Ile Leu Glu Trp Glu Asp Arg Glu Gln Gly Ile Phe Arg  
 180 185 190  
 Val Val Lys Ser Glu Ala Leu Ala Lys Met Trp Gly Gln Arg Lys Lys  
 195 200 205  
 Asn Asp Arg Met Thr Tyr Glu Lys Leu Ser Arg Ala Leu Arg Tyr Tyr  
 210 215 220  
 Tyr Lys Thr Arg Ile Leu Glu Arg Val Asp Arg Arg Leu Val Tyr Lys  
 225 230 235 240  
 Phe Gly Lys Asn Ala His Gly Trp Gln Glu Glu Lys Leu  
 245 250

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCCAGTCTTG GTCTCTTCAG CATC

24

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGAGATGCA GTTGGCATCA AGCT

24

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## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCCAGTGTT ATGGGTGCTG

20

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACAGTCACTT GATCCACGGC CAATCC

26

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

CCTGTGACTC ATACTGGACA TC

22

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTTGTGTGCG GATGTTCTGG

20

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(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGGGATCCTT GGACTCCGTA ACCCATAGC

29

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCAGATCTCA GAGTTTCTCT TCCTGCC

27

DATED this 30th day of September 1998

Monash University  
by their Patent Attorneys  
DAVIES COLLISON CAVE

atcacacggctacaggtgcctttatttctacagtcgctggtgctgggagcgcgct	56
tgccttctcttgccctgaaagccttctgtctggac	91
ctagccaccacttgtcttcacgggtg	116
<b>ATGTTGGACTCCGTAACCCATAGCACCTTCCTGCCCAACGCATCCTTCTGTGACCCCCTG</b>	176
ATGCCTTGGACCGATCTGTTTACGAATGAAGACTACTACCCTGCCTTTGAGCATCAGACA	236
GCCTGTGATTCTACTGGACATCAGTGCACCCTGAATACTGGACCAAGCGCCATGTCTGG	296
GAATGGCTCCAATTCTGCTGTGACCAGTACAAGCTTGATGCCAACTGCATCTCCTTCTGT	356
CACTTCAACATCAGCGGCCTGCAGCTCTGCAGCATGACGCAGGAGGAGTTCATTGAGGCA	416
GCCGGCATCTGTGGGGAGTACCTGTACTTCATTCTCCAGAACATTCGCTCGCAAGGTTAC	476
TCCTTTTTCAATGATGCTGAAGAGACCAAGACTGGCATCAAAGACTATGCTGATTCCAGT	536
TGCTTGAAAACAAGTGGCATCAAGAGTCAAGACTGTCACAGCCGAACAAGCCTCCAAAGT	596
TCTCACCTGTGGGAATTTGTTCAGAGACTTGCTGCTGTCCCCTGAAGAGAACTGTGGCATC	656
CTGGAATGGGAAGACAGGGAGCAGGGCATTTCAGAGTGGTTAAGTCAGAAGCCCTGGCA	716
AAGATGTGGGGACAAAGGAAGAAGAATGACAGGATGACGTACGAGAAGCTGAGCCGAGCC	776
CTGAGATACTACTATAAAACGAGAATTCTGGAGCGGGTTGACCGGAGGTTAGTGTACAAA	836
TTTGGAAAGAACCGGCACGGGTGGCAGGAAGAGAACTCTGA	896
ctcatttgatggatttctgttgttgaaacaatcagatcaaactagacatttgaaagtct	956
ccctcctcctcctcctccccctccttccccctccttcttcttccccctcctcctcttca	1016
aaacctacaaacacactgataaaatttctgcatgtctcagcttacatttgaaattcagttg	1076
ttgtctatttggggcgatgccatcagcccttaagcaatcgtcttcatcccaagggggagga	1136
agggatggcttctgtggcaacttgggtgtgacactgtctccttaatgaagtgttggagcta	1196
agggagccagtggttatgggtgctgtttcacaagaggaccggtgcaccattaagacacat	1256
gatcctccccgttccaggggttctgagcgggtcgactgaggcagcttgccctgtggttagttt	1316
ttaggaaagggagatgtaagacttcccttgctttagatttgaaattatcacagttatattc	1376
catagaagaattttttaattgaaaaaatttttagtggttaagccactaaactgggacctaat	1436
tggatgtagcctaagtttactaataagtttcttaaccagatcaccatttccaaccacttagc	1496
cacagtcacttgatccacggccaatccttctg	1528
aacttaacatccttgtagtttagtcacct	1556
tgggaattgctacctagattgttacccttcaacctcactgggtggctatcatcaggtcta	1616
cagtacctgatcaacagacatgtgcatttaatttctaaatcactgctgtgcctatgattc	1676
aaaccgtcagcgtgttcagtttattgattctctctgaggtcggaatttatgattctctc	1736
tgaggctaagacattaaacctttaccaagcagagaacgtcctaacaagccacgatagccg	1796
aacacagcatcgatctcttctcttttctgatgaatactcaaactttccaacatattctct	1856
tcacaaaagtaaaagacagtgaatttatcaatcaacgttcatgggttaaagtctgcact	1916
gacatttccctgtctgccgttgcatgccgttggcatgcaaggtgttaatgacctgcaaca	1976
tggtggagtgccctgaaccctaacttccccagagttgggactgtctagtgaccggcactg	2036
aatagcaatgcaggctgaagacctccaggtttagaatttaacctcaaaagtaacttggtt	2096
ttaaaaagaaatgtgaattactgtaaaataatctatttttggattcgtgtgtttttcagg	2156
tggatatagtttataaacaatgtgaataaaaaatatttatcatgtttaaaaaaaaaaaaa	2216
aaaaaaaa	2224

FIGURE 1a

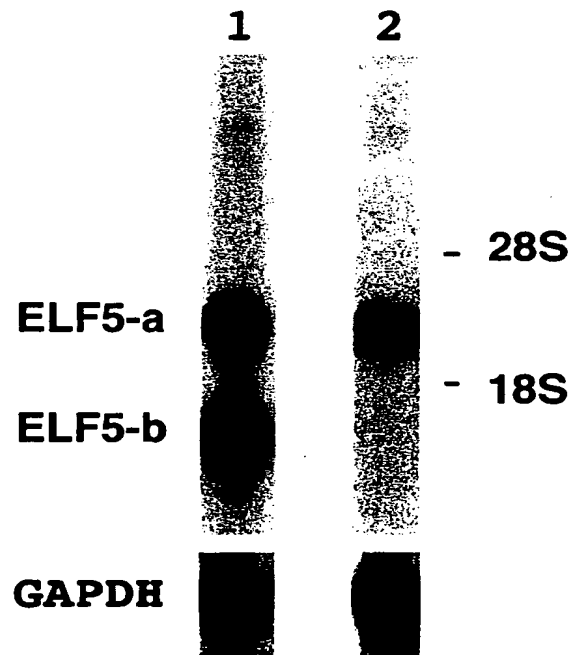


FIGURE 1b

hELF5	MLDSVTHSTFLPNASFC	DPLMSWTDLFS	NEEYYP	AFEHQ	TACDSYWTSVH	50
mELF5	MLDSVTHSTFLPNASFC	DPLMPWTDLFS	NEDYYP	AFEHQ	TACDSYWTSVH	50
	<div>CKII</div> <div>CKII</div> <div>CKII</div>					
hELF5	PEYWK	KRHVWEWLQFCCDQYK	LDTNCISFC	NFNISGLQ	LCSMTQEEFVEA	100
mELF5	PEYWK	KRHVWEWLQFCCDQYK	LDA NCISFC	HFNISGLQ	LCSMTQEEFIEA	100
	<div>PKC</div> <div>CKII</div>					
hELF5	AGICGEYLYFILQ	NIRTOGYSFF	NDAEESK	ATIKDYAD	SNCLKTSGIKSQ	150
mELF5	AGICGEYLYFILQ	NIRSQGYSFF	NDAEETK	TGIKDYAD	SSCLKTSGIKSQ	150
hELF5	DCHSHSRTSLQSSHL	WEFVRDLLIS	PEENCGILEWED	REQGIFRVVKSEA	200	
mELF5	DCHS--RTSLQSSHL	WEFVRDLLIS	PEENCGILEWED	REQGIFRVVKSEA	198	
	<div>CKII</div>					
hELF5	LAKMWGQRKKND	RMITYEKL	SRALRYYYKT	GILERVDRRLVYKE	GKNAHW	250
mELF5	LAKMWGQRKKND	RMITYEKL	SRALRYYYK	TRILERVDRRLVYKE	GKNAHW	248
	<div>TyP</div>					
hELF5	QEDKL*					255
mELF5	QEEKL*					253

FIGURE 2a

## IDENTITY

(%)

hELF5	LWEIFVRLDLSPE-ENCGILEWEDREQGIFRVV--KSEALAKMWGQRK-KNDRMTYEKLSRALRYYYKTGILERVD--RRLVYKF	100
mELF5	LWEIFVRLDLSPE-ENCGILEWEDREQGIFRVV--KSEALAKMWGQRK-KNDRMTYEKLSRALRYYYKTGILERVD--RRLVYKF	98
hELF3	LWEIFRDLIHPE-LNEGLMKWENRHEGVFKEL--RSEAVAQLWGQKK-KNSNMTYEKLSRAMRYYYKREILERVD--GRLVYKF	67
mELF3	LWEIFRDLIHPE-LNEGLMKWENRHEGVFKEL--RSEAVAQLWGQKK-KNSNMTYEKLSRAMRYYYKREILERVD--GRLVYKF	67
hNERF	LWFFLLDQLQDN--TCPRIYIKWTQREKGIFKLV--DSKAVSKLWGKHK-NKPDNMYETMGALRYYYQRGILAKVE--GORLVYQF	49
dETS4	LWQFLKELLASPO-VNGTAIRWIDRSKGIFKIE--DSVRVAKLWGRRK-NRPAMNYDKLSRSIRQYKKGIMKKTTERSQRLVYQF	48
dE74A	LWFFLLKLLQDRE--YCPRFIKWTNREKGVFKLV--DSKAVSRLWGMHK-NKPDNMYETMGALRYYYQRGILAKVD--GORLVYHF	48
hELF1	LWFFLLALLQDKA--TCPKIYIKWTQREKGIFKLV--DSKAVSRLWGMHK-NKPDNMYETMGALRYYYQRGILAKVE--GORLVYQF	46
hELK1	LWQFLLOLREQ--GNGHIIISWTSRDGGEFKLV--DAEEVARLWGLRK-NKTNNMYDKLSRALRYYYDKNIIRKVS--GQKFVYKF	46
hTEL	LWDYVYQLLSDS--RYENFIRWEDKESKIFRIV--DPNGLARLWGNHK-NRTNMTYEKMSRALRHYYYKLNIIIRKEP--GORLLFRF	44
hERM	LWQFLVTLDDP--ANAHFIAWTGR-GMEFKLI--EPEEVARRWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA--GERYVYKF	44
mER81	LWQFLVALLDDP--SNSHFIAWTGR-GMEFKLI--EPEEVARRWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA--GERYVYKF	44
mPEA3	LWQFLVALLDDP--TNAHFIAWTGR-GMEFKLI--EPEEVARRWGIQK-NRPAMNYDKLSRSLRYYYEKGIMQKVA--GERYVYKF	44
mGABPa	LWQFLLELLTDK--DARDCISWVGDEG-EFKLN--QPELVAQKWGQRK-NKPTNMYDKLSRALRYYYDGMICKVQ--GKRFVYKF	44
mERP	LWQFLLELLTDK--KHEHLICWTSNDG-EFKLI--KAEVAKLWGLRK-NKTNNMYDKLSRALRYYYDKNIIRKVI--GQKFVYKF	42
dETS6	LWQFLLELLADS--SNANAISWEGQSG-EFKLI--DPDEVARRWGERK-AKPNNMYDKLSRALRYYYDKNIMTKVH--GKRYAYKF	42
mPU1	LYQFLLDLLRS--DMKDSIWWVDKDKGTQFSSKHKEALAHRWGIQKGNRKMTYQKMARALRNYGKTGEVKKVK--KKLTYQF	42
hPE1	LWHFILELLQKE--EFRHVIAWQQGEYGEFVIK--DPDEVARRWGERK-CKPQNNYDKLSRALRYYYNKRILHKTG--GKRFTYKF	42
hSAP1	LWQFLLOLLQKP--QNKHMICWTSNDG-QFKLI--QAEVARRWGIQK-NKPNMYDKLSRALRYYYVKNIIKKVN--GQKFVYKF	42
hSPIB	LYQFLLGLLTRG--DMRECVMWVEPGAGVQFSSKHKEALLARRWGQKGNRKMTYQKLARALRNYAKTGEIRKVK--RKLTYQF	42
dYAN	LWDFLOQLLNDRNQKYSDLIAWKCRTDGVFKIV--DPAGLAKLWGIQK-NHLSMNYDKMSRALRYYYRVNLRKVQ--GERHCYQF	41
hERG	LWQFLLELLSDS--SNSCITWEGTNG-EFKMT--DPDEVARRWGERK-SKPNNMYDKLSRALRYYYDKNIMTKVH--GKRYAYKF	41
mFLI1	LWQFLLELLSDS--ANASCITWEGTNG-EFKMT--DPDEVARRWGERK-SKPNNMYDKLSRALRYYYDKNIMTKVH--GKRYAYKF	41
dELG	LWQFLLEILLTDK--EHTDVIEWVGTEG-EFKLT--DPDEVARRWGERK-NKPNMYDKLSRALRYYYDGMICKVS--GKRFAVYKF	40
dETS3	LWQFLLELLSDS--NNASCITWEGTNG-EFKLT--DPDEVARRWGERK-SKPNNMYDKLSRALR-----	39
mETS1	LWQFLLELLTDK--SCQSFIWTDGW-EFKLS--DPDEVARRWGERK-NKPKMNYEKLRSGLRYYYDKNIIHKTA--GKRYVYRF	37
mETS2	LWQFLLELLSDK--SCQSFIWTDGW-EFKLA--DPDEVARRWGERK-NKPKMNYEKLRSGLRYYYDKNIIHKTS--GKRYVYRF	37
mER71	LWQFLLELLQDG--ARSSCIRWTGNSR-EFQLC--DPKEVARLWGERK-RKPGMNYEKLRSGLRYYYRRDVLKSG--GRKYTYRF	36
consensus	LWQFLLELLD I W FK VAR WG K P MNY KLSR LRYYY I K GR Y F	

FIGURE 2b



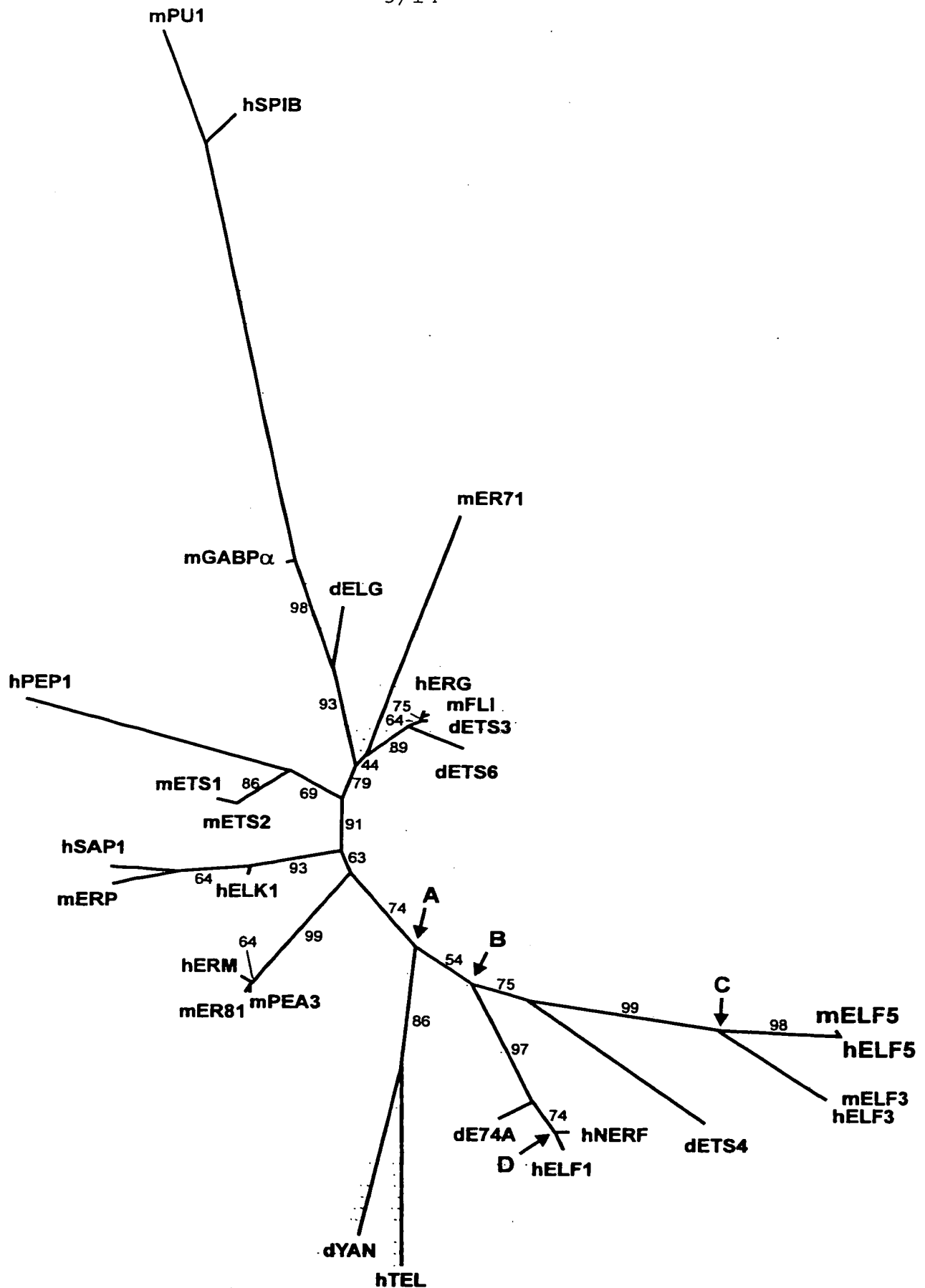


FIGURE 2c

[illegible]

FIGURE 2d

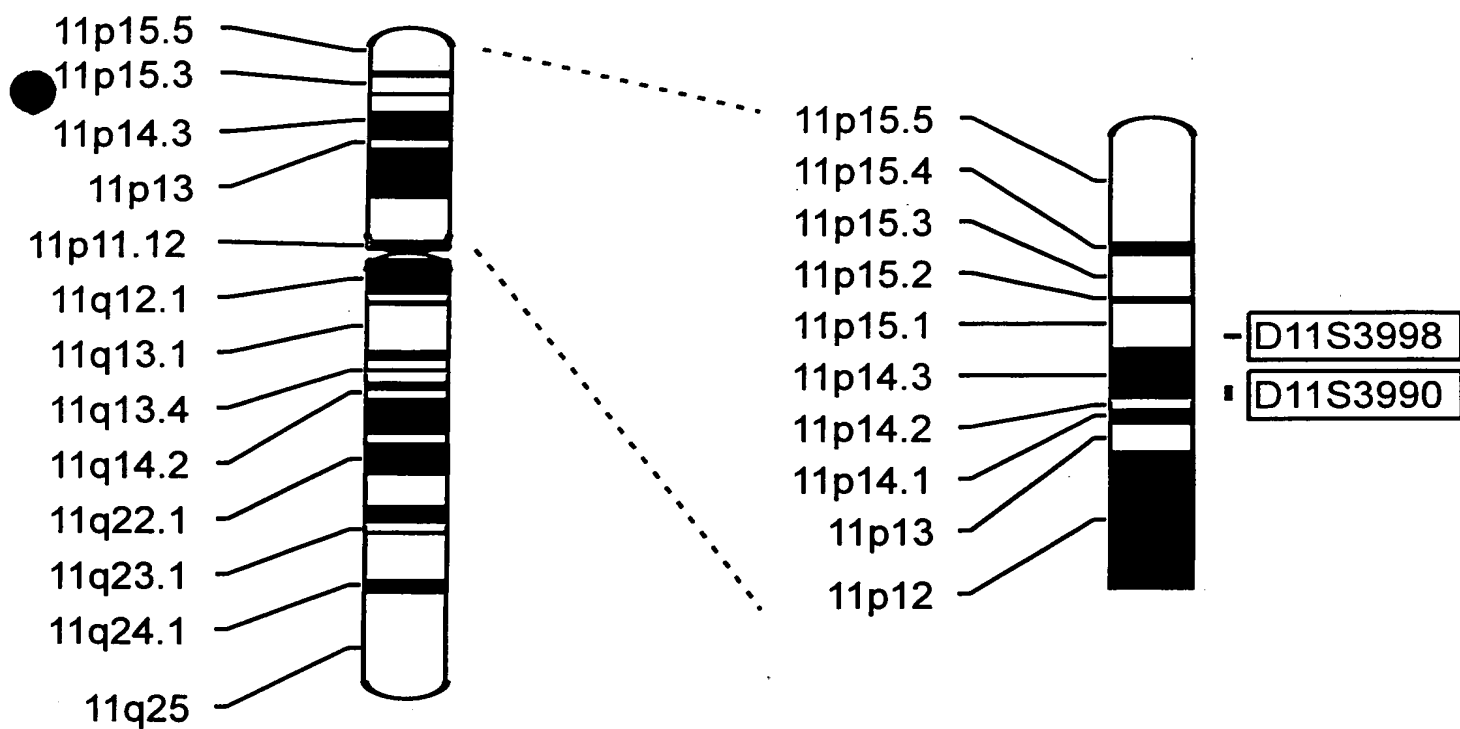


FIGURE 3

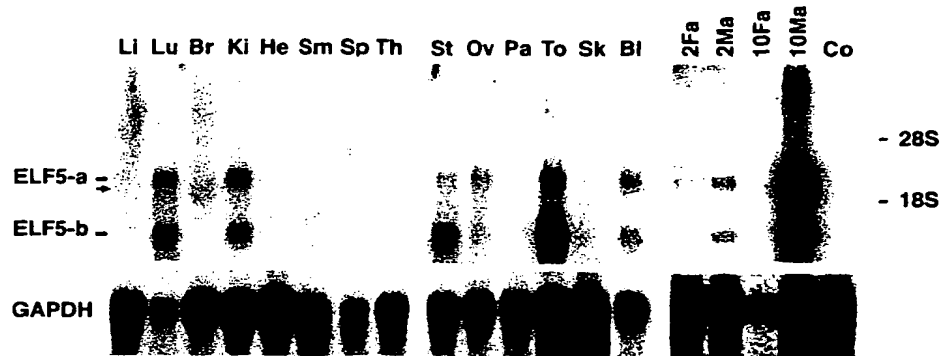
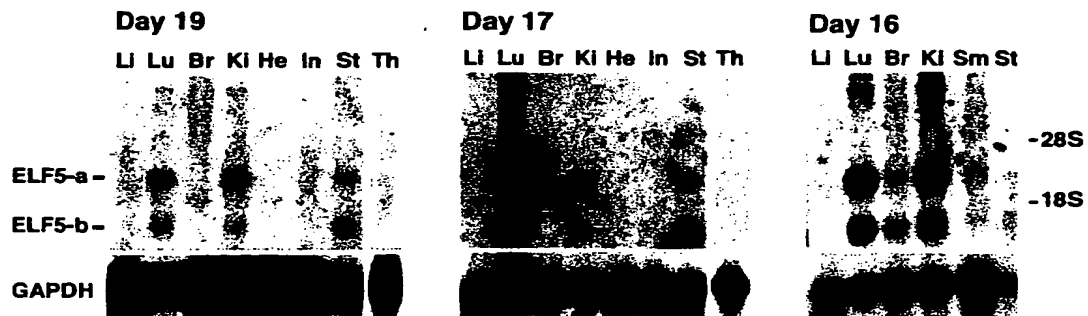
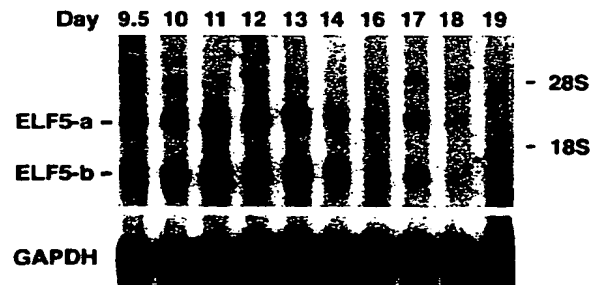
**a****b****c****d**

FIGURE 4

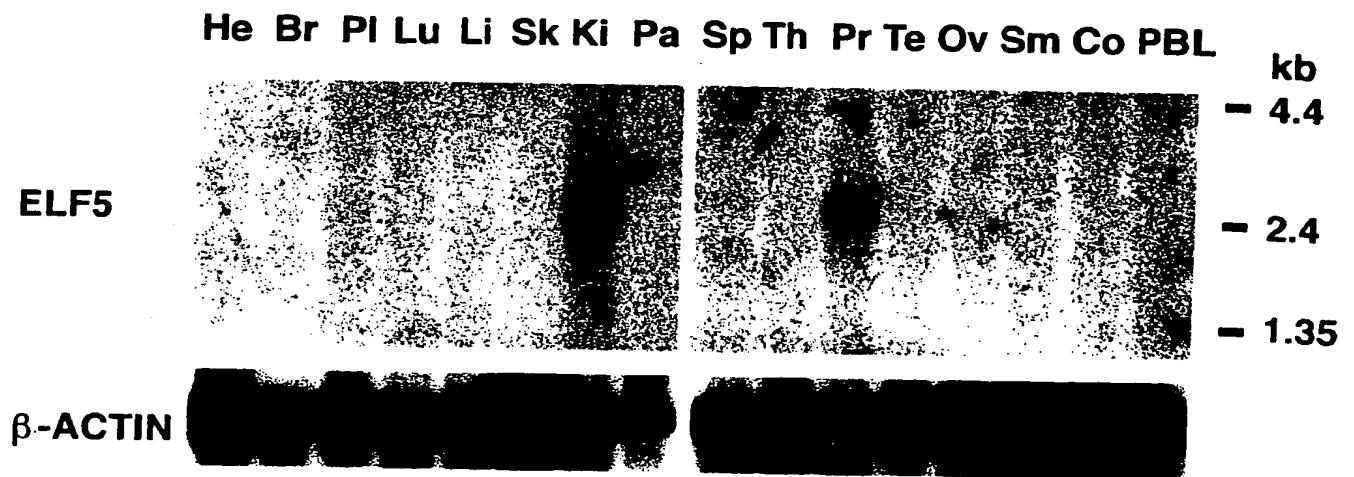
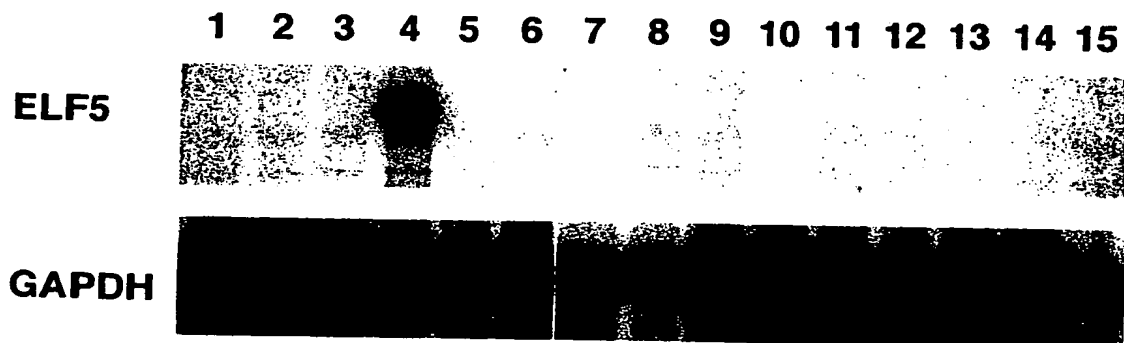
**a****b**

FIGURE 5

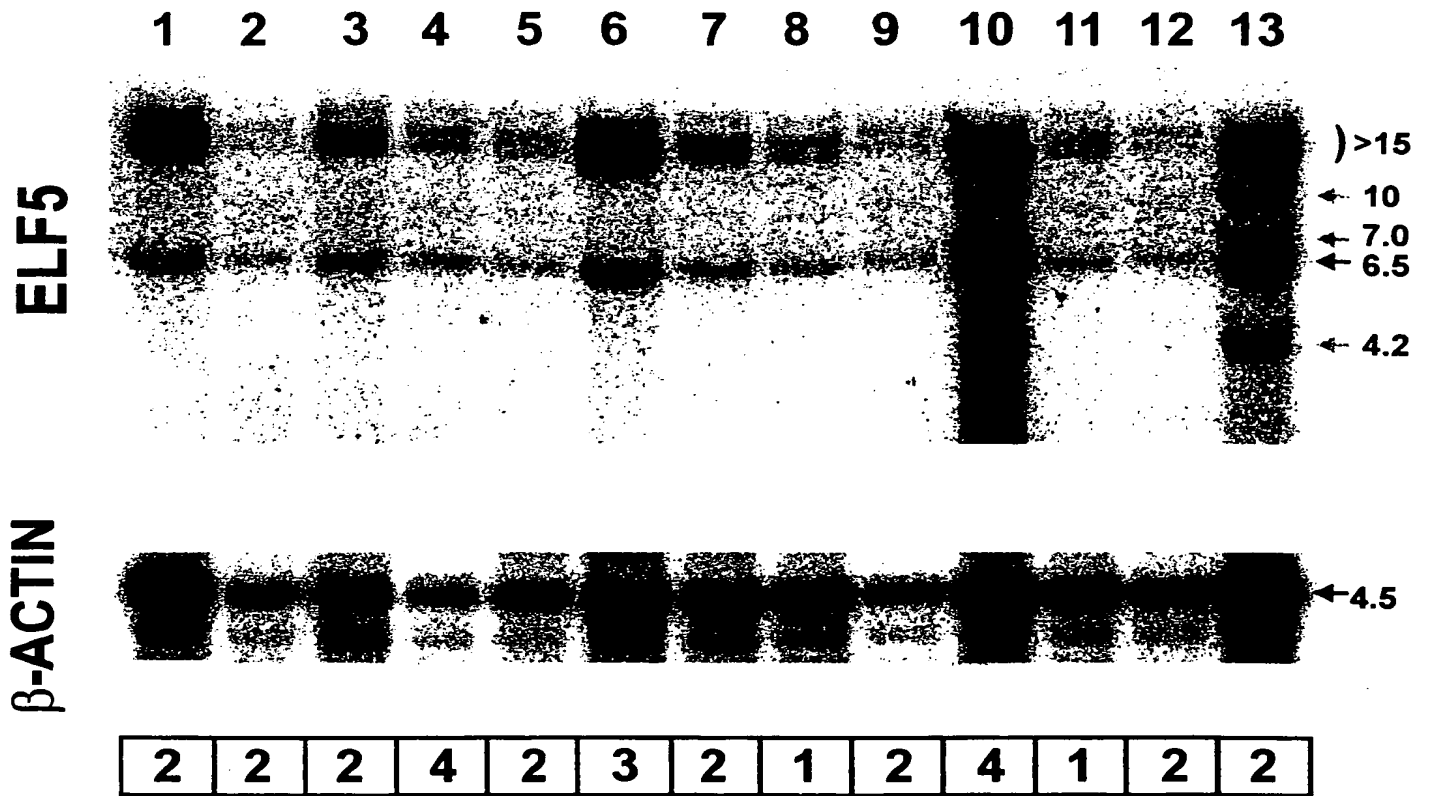
**C**

FIGURE 5c

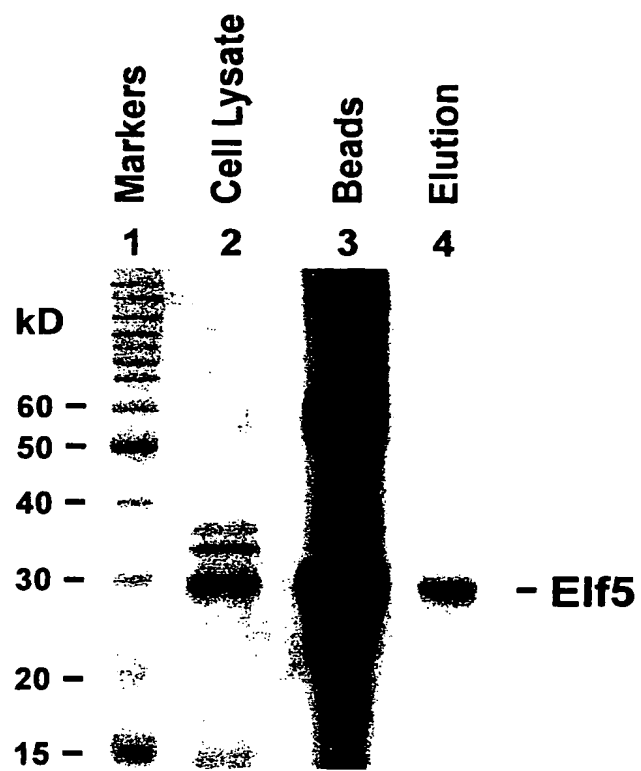


FIGURE 6a

Protein	Elf5								ETS1	
Probe	E74	E74 m1	E74							
Competitor	-	-	-	E74	E74 m1	GM ETS	ERB B2	MSV	AP1	-
	1	2	3	4	5	6	7	8	9	10

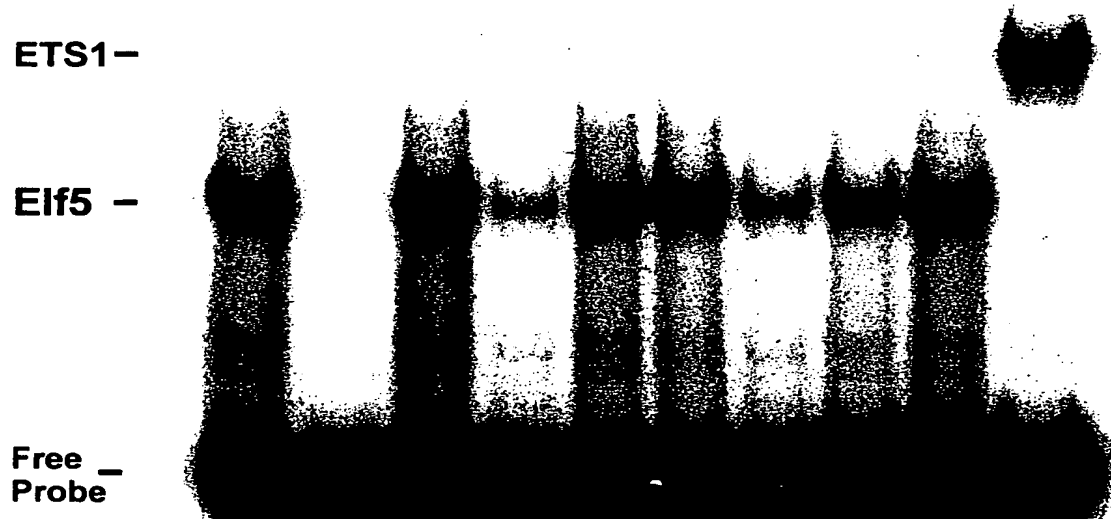


FIGURE 6b



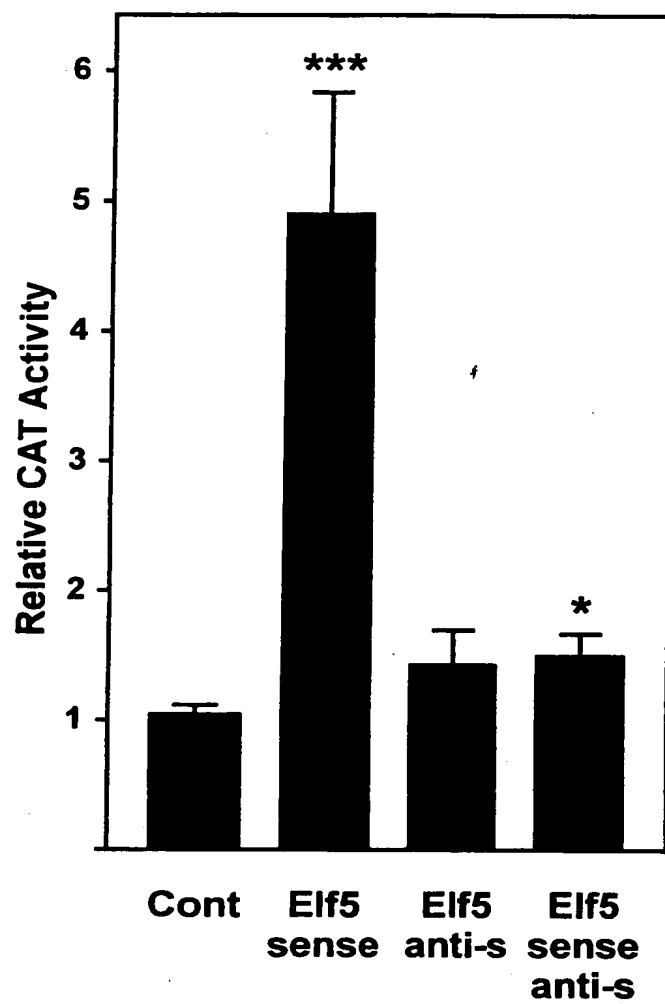
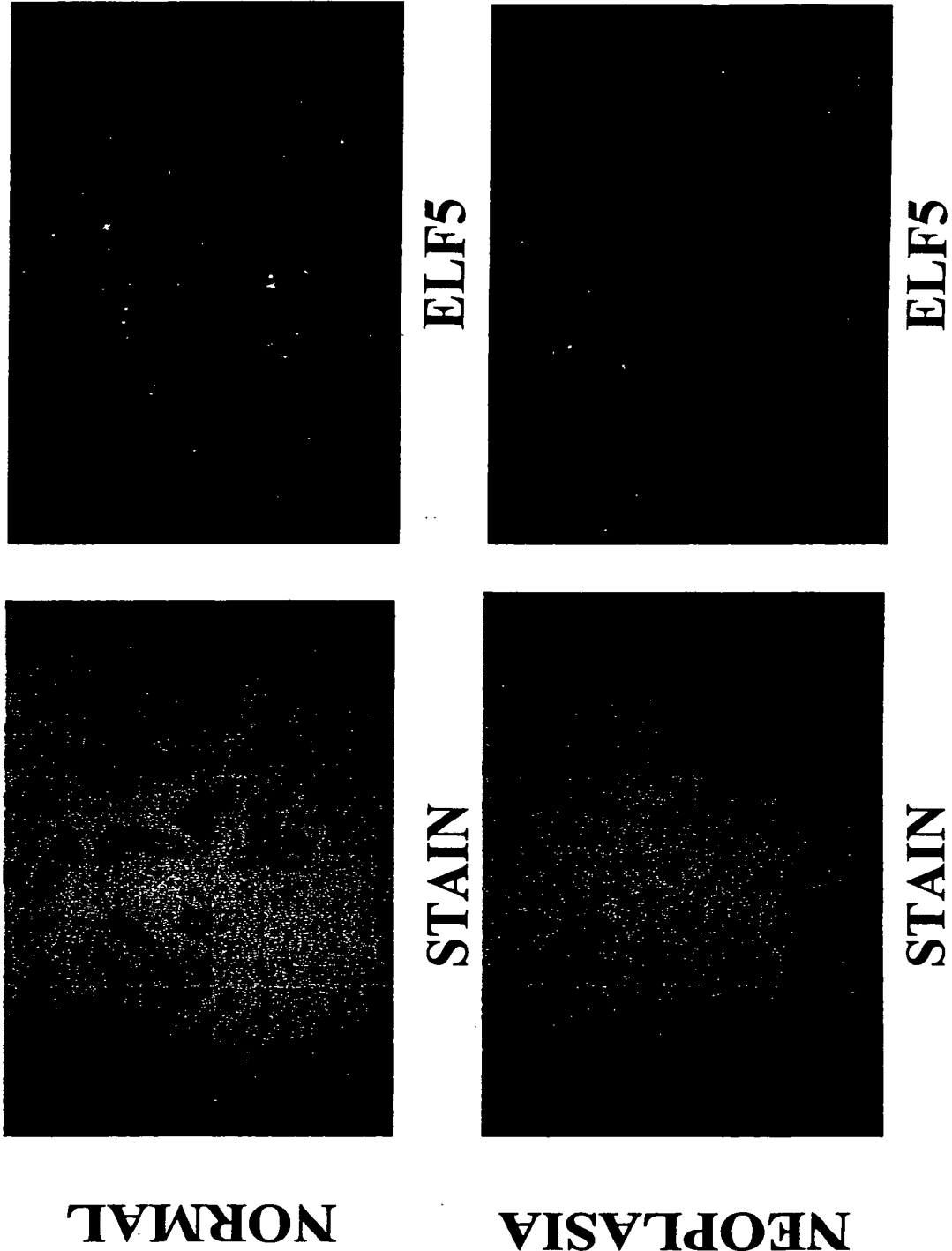


FIGURE 7

**ELF5 expression is not detectable in human primary breast cancer cells, but is strongly expressed in adjacent normal epithelium**



**FIGURE 8**